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ABSTRACTS

Babson, A. L., Read, P. A., and Phillips, G. E.: The importance of the substrate in assays of acid phosphatase in serum. Am. J. of Clin. Path. 32:1 83-87, July 1959.

A comparison is made between six generally used substrates for their relative specificity to prostatic and erythrocytic acid phosphatase. Results showed that a new substrate, alpha-naphthyl phosphate, was twice as specific as beta-glycerophosphate and 40-100 times as specific as all other substrates investigated. Acid phosphatase assays in serum should be specific for that enzyme arising from cancerous prostatic tissue. The substrate alpha-naphthyl phosphate* is specific for this enzyme.

Babson, A. L., and Read, P. A.: A new assay for prostatic acid phosphatase in serum. Am. J. Clin. Path. 32:1, 88-91, July 1959.

A new and specific method for the determination of prostatic acid phosphatase in serum is presented. The method is based on the use of a new substrate alpha-naphthyl phosphate which measures only prostatic acid phosphatase. The method is simple and rapid to perform with a minimum of manipulation. In this procedure the color developed represents prostatic enzyme activity alone. The reagents for this new method are available in convenient and stable form.*

TABLE OF CONTENTS

VOLUME 69 APRIL 1960 NUMBER 4

ORIGINAL ARTICLES

REGULAR DEPARTMENTS

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VOLUME 69 APRIL 1960 NUMBER 4

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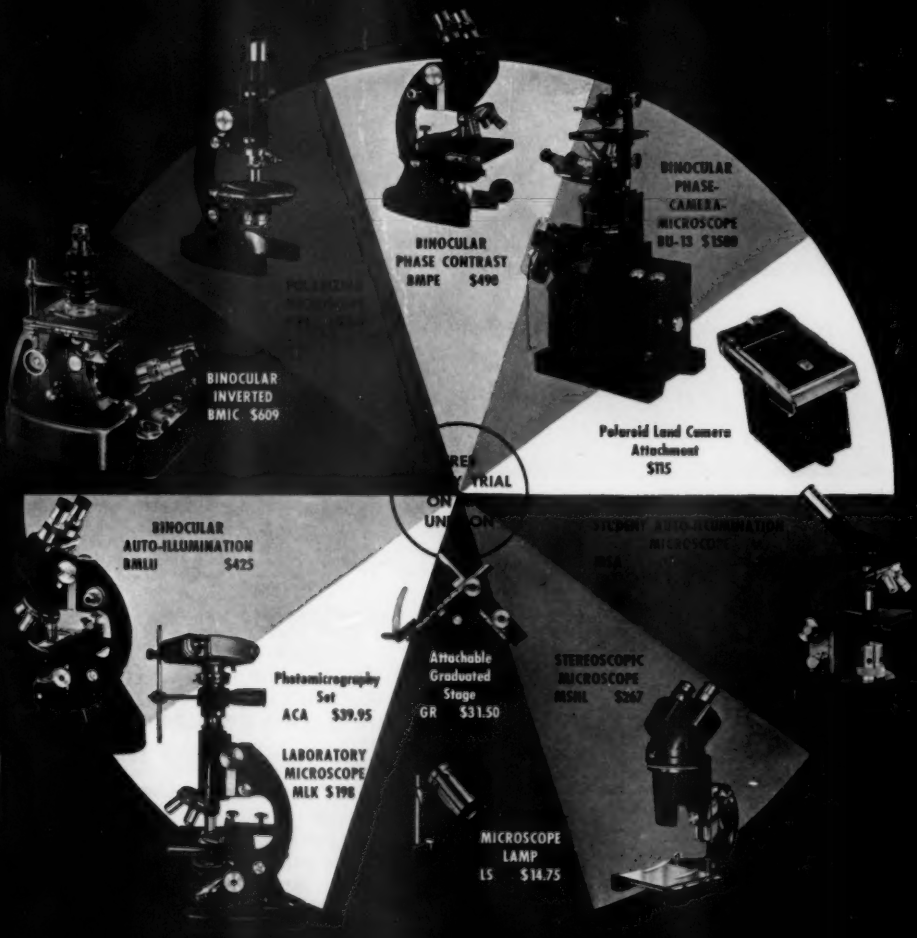

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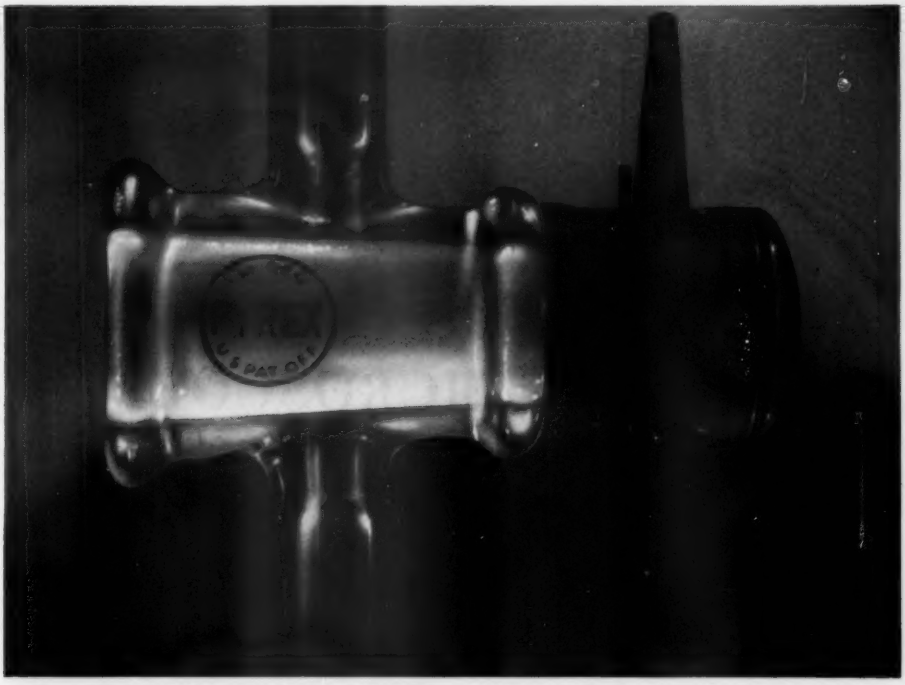

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Cerebral Vascular Lesions and Peptic Ulceration

JØRGEN B. DALGAARD, M.D., Finsengade, Aarhus, Denmark

This paper is part of a more comprehensive study of so-called neurogenic peptic ulcerations as seen at necropsy. In previous papers the usual types of neurogenic lesions of the upper part of the gastrointestinal tract, acute esophagogastroduodenal ulcers, and so-called malacias (which are agonal or early postmortem perforations of the esophagus or stomach) have been described,[6] and the following cerebral lesions prone to induce such complications have been considered: cerebral injuries,[7] tumors,[8] operations,[10] and infections.[11] The influence of acute severe stress,[28] notably severe burns,[9] and of corticosteroid therapy, hypoxia, and intoxications [28] on the pathogenesis of these lesions should also be mentioned. The aim of this paper is to analyze cases of ulcer associated with cerebral vascular lesions which in my material proved to be the commonest single cause of acute peptic ulceration.[29]

The literature on this particular subject is rather sparse. Apart from publications by the Hungarian pathologist Joseph Baló,[1-3,12] I know of no papers specially devoted to this problem. Several papers on other aspects of neurogenic peptic ulceration, however, contain reports of isolated cases of the type here considered.

Pomorski,[21] in 1892, described gastric ulcer and erosions in a newborn with intracranial hemorrhage. Similar cases have been reported by others. Lépine,[17] in 1895, mentioned a case of fatal hemorrhage from a gastric ulcer secondary to cerebral hemorrhage in an adult. Hart [16] reported three similar cases. The 22 cases of neurogenic peptic ulceration reported by Opper and Zimmerman [20] include 5 with cerebral hemorrhage or infarction and 1 with ruptured intracranial aneurysm.

Baló,[1] in 1941, encountered 14 cases of major cerebral hemorrhage among 118 necropsies on ulcer patients. The number of other cerebral lesions was not mentioned. None of the 14 ulcers was of the chronic type. Comparative evaluation of the ages of the cerebral and gastrointestinal lesions indicated a causal relationship. The frequency of peptic ulceration in cases of fatal cerebral apoplexy was found to be 20%, but it was stressed that most patients with apoplexy die before sufficient time has elapsed for an ulcer to develop; in other cases superficial ulcers might heal before the fatal outcome. Baló concluded that cerebral apoplexy may cause peptic ulceration in the esophagus, stomach, and duodenum, and that such lesions are acute and may perforate or heal, but usually do not develop into chronic ulcers. The investigation was later [2,12] extended to 240 cases of ulcer, in 12% of which there were large hemorrhages in the basal nuclei, while in an additional 20% punctate hemorrhages were found in or near the hypothalamus. Recently, Baló [3] maintained the view that both acute and chronic peptic ulcers occur

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through nervous system irritation of various durations.

Strassmann[24] studied the occurrence of neurogenic peptic ulceration in 1,400 medicolegal and mental-hospital necropsies. Of 26 acute ulcers and 30 perforating esophageal or gastric malacias, all except 2 were associated with intracranial lesions, 14 of which were of acute vascular origin. A few additional cases were included in a subsequent paper.[25] The observations were indicative of an etiologic relation between the cerebral and the peptic lesions effected through a neurovascular mechanism, as suggested by Cushing.[5]

Globus and Ralston,[14] studying esophagogastroduodenal erosions and ulcers in relation to disorders of the nervous system, observed five cases following cerebral vascular lesions. A similar case was included in Bsteh's[4] neurosurgical series. Gastromalacia associated with carotid-artery thrombosis with extensive cerebral infarction was observed in two patients by Watson and Netsky.[26] Additional isolated cases of fatal upper gastrointestinal perforation following cerebral hemorrhage have been reported by others.[13,15,18,19,22,27]

Spencer[23] analyzed five-year necropsy material from the Mayo Clinic with regard to lesions of the upper part of the gastrointestinal tract that were associated with intracranial neoplasms. The results obtained from statistical evaluation were identical with those concomitantly achieved by me.[8] Of special interest here, however, is Spencer's observation that gastrointestinal lesions were present in 60% of her 87 cases of intracranial neoplasms with associated hemorrhage.

Material

The basic material consists of 4,317 necropsies. Of these, 1,500 originated from each of the university institutes of pathology of Aarhus, Denmark, and Bergen, Norway, and were for the main part performed or supervised by me. The remaining 1,317 were medicolegal necropsies from the same institutes and from the Institute of Legal Medicine, University of Copenhagen, Denmark.

A total of 208 cases of acute and 177 cases of chronic peptic ulcers, including sequelae, was revealed. Details concerning the basic material, with description and classification of types of ulceration, were given in a previous paper,[8] which also contained a grouping of the cerebral lesions found in the ulcer cases. The main single group was that of cerebral vascular lesions, including spontaneous subarachnoid hemorrhage, acute intracerebral hemorrhage or thromboembolic infarction, and localized recent or old cerebral infarcts (softenings), in Scandinavia often termed "emollitions." Cases in which there was hemorrhage secondary to cerebral injuries, neoplasms, or operations are excluded here.

Exactly 100 necropsies, or 2.3% of the basic material, revealed both cerebral vascular lesions and esophageal, gastric, or duodenal ulceration. These cases are the subject of the present study.

Results

In the following analysis, cases of acute and cases of chronic peptic ulceration will be considered separately. The acute cases are furthermore divided into subgroups according to the type of cerebral vascular lesion (Table 1).

I. Cases of Acute Peptic Ulceration and Cerebral Vascular Lesion.—Acute Spontaneous Subarachnoid Hemorrhage: Thirteen patients (Table 2) had necropsy findings of sudden severe subarachnoid hemorrhage, which was clinically diagnosed in most cases. The ages of these patients ranged from 20 to 77, with an average of 49 years; eight were male and five female. Ten had ruptured basal aneurysms; three of these and two of the remaining patients were known to be hypertensive.

The duration of the acute cerebral symptoms was less than one week in six cases

TABLE 1.—*Survey of One Hundred Cases of Cerebral Vascular Lesions Associated with Ulceration of the Upper Part of the Gastrointestinal Tract*

Type of Vascular Cerebral Lesion	Type of Peptic Ulceration	
	Acute	Chronic
Acute subarachnoid hemorrhage	13	0
Acute cerebral hemorrhage	30	9
Acute thromboembolic infarction	7	2
Cerebral infarct ("emollition") without gross hemorrhage or thrombosis	17	22
Total cases	67	33

TABLE 2.—*Acute Spontaneous Subarachnoid Hemorrhage with Associated Acute Peptic Ulceration* *

Case No.	Sex	Age	Main Course; Survival Time; Intracranial Pathology	Esophagogastroduodenal Lesion (Time Death to Necropsy)	Group
202	M	59	Hypertensive, found unconscious; death within 24 hr.; extensive subarach. hemorrhage from ruptured aneurysm on r. middle cereb. artery	Multiple acute gastric erosions (44 hr.)	C
340	M	42	Found unconscious; death 30 hr. later; very large subarach. hemorrhage from ruptured aneurysm on l. int. carotid artery	Perforating gastromalacia; duodenal contents hemorrhagic (23 hr.)	B
178	F	56	Admitted for severe hypertension, sudden subarach. hemorrhage; died 2 days later; no aneurysm found; adrenocortical carcinoma	Gastromalacia with 3 perforations, hemorrhagic mucosa; Inflammatory reactions on adjacent organs (14 hr.)	B
216	M	53	Hypertensive with ruptured aneurysm on ant. commun. artery; extensive subarach. hemorrhage, invading brain; died on 3d day	Large perforating esophagomalacia (4×1.5 cm.) with vital reactions (8 hr.)	A
53	M	46	Spontaneous large subarach. hemorrhage from ruptured aneurysm on l. middle cereb. artery; craniotomy with difficult hemostasis on 1st; death on 5th day	One old and 3 acute duodenal ulcers up to 30×15 mm. Tarry stools (25 hr.) (Fig. 1).	E E chr.
23	M	20	Spontaneous subarach. hemorrhage from ruptured aneurysm on post. commun. artery; operation difficult; death on 6th day	Multiple gastroduodenal erosions (12 hr.)	C
74	M	60	Sudden subarach. hemorrhage from ruptured aneurysm in r. carotid angle; death on 8th day; hemorrhage invaded r. hemisphere	Esophagomalacia with 7×2 cm. perforation; 400 ml. hemorrhagic gastric acid in r. pleural cavity (19 hr.)	A
200	F	46	Hypertensive with sudden subarach. hemorrhage from ruptured aneurysm on r. mid. cereb. artery; invasion of r. hemisphere; death 2 wk. after onset of acute symptoms	Acute duodenal ulcer	E
71	M	40	Polycystic kidneys and hypertension; spontaneous subarach. hemorrhage with symptoms at first subsiding, then suddenly worsened, with death on 15th acute day; no aneurysm found	Multiple gastric erosions (17 hr.)	C
356	M	57	Found unconscious; considered nonoperable; died on 19th day; extensive subarach. hemorrhage from ruptured basal aneurysm	Fairly large, superficial ulcer (scar?) in duodenum (see "Comment") (31 hr.)	E (chr.?)
62	F	39	Sudden subarach. hemorrhage with symptoms gradually subsiding; ruptured aneurysm r. ant. cereb. artery; operation on 27th day complicated by large hemorrhage; death 3 days later	Gastromalacia (4×1 cm.) perforated to peritoneal cavity; perforating esophagomalacia (2×1 cm.) concealed by pleural adhesions (20 hr.)	A B
33	F	43	One mo. severe headache with dizziness, double vision, and ultimately blindness; subtemporal decompression without effect; death next day; subarach. hematoma and cerebral herniation; no visible aneurysm	Gastromalacia perforating at necropsy (46 hr.)	B
167	F	77	Within 2 mo. 3 episodes with symptoms of subarach. hemorrhage; last attack fatal in few hours; ruptured aneurysm on l. mid. cereb. artery	Large gastromalacia with 10 cm. perforation and mucosal hemorrhages (9 hr.)	B

* Thirteen cases listed according to duration of cerebral symptoms, with intracranial and gastrointestinal findings indicated. The lapse of time from death to necropsy is indicated. The grouping refers to that used in the other papers in the series.

and exceeded a month in only one case, in which three acute cerebral attacks had occurred within the last two months. Excluding this case, the average interval between onset of symptoms and death was 10 days. The ruptured aneurysms were situated two on each middle cerebral artery, one in each carotid triangle, and two on the anterior, and one on the posterior, communicating branch, and the site of one was not exactly stated. Four were on the right side, and three on the left side, and the remainder were in the midline.

The types of gastrointestinal ulceration will be considered with the following subgroups. In one case both acute and chronic ulceration were displayed (Fig. 1). In another case (Case 356) the lesion was first considered to be a scar, but then was determined to be a superficial ulcer already in a state of healing. In no case had the ulceration been diagnosed during life, although

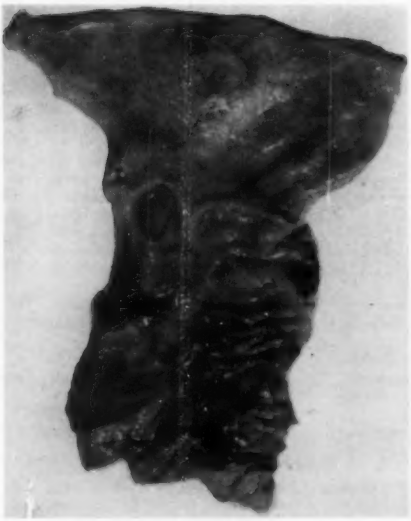

Fig. 1 (Case 53).—Three large acute duodenal ulcers from a 46-year-old man with a large subarachnoid hemorrhage from a ruptured basal aneurysm. Craniotomy with difficult hemostasis was performed on the first day, and death occurred on the fifth day after rupture. The intestinal contents were bloody. A pyloric scar was also present. There was no history of ulcer.

the necropsy findings indicated that the ulcerations were, in some cases at least, a contributing cause of death.

Acute Cerebral Hemorrhage and Thromboembolic Infarction (Tables 3 and 4 and Figs. 2 to 4): These cases were instances of clinically typical cerebral apoplexy and may conveniently be considered together. Of the 30 patients with hemorrhage, 20 were men, and of the 7 with thrombosis, 5 were men. The ages varied from 23 to 77, with an average of 56 years, in the first subgroup, and from 43 to 77, with an average of 60 years, in the second subgroup. Eighteen patients with hemorrhage and three with thrombosis were known to be hypertensive; three had polycystic kidneys, and three were diabetics. In two patients the cerebral hemorrhage was an ultimate complication of acute leukemia. Three patients were found dead, in one of whom (Case 171) the disease process was complicated by barbiturate intoxication. Two patients also had chronic ulcers.

The duration of the severe acute cerebral symptoms was usually short; in eight cases of hemorrhage the duration was less than 12 hours, and in half of all cases it did not exceed 24 hours. Only five patients survived the first week (maximum three weeks). Mean survival time was four days. The sites of the cerebral lesions varied.

The acute peptic lesion was not diagnosed in any case. In one case (Case 46) the perforating ulcerations must be considered the ultimate cause of death; in other cases, ulcerations were possibly contributing causes of death.

Acute and Subacute Cerebral Infarcts (Softenings) Without Gross Hemorrhage or Thrombosis: Of the 17 patients in this group (Table 5 and Fig. 5) 10 were men and 7 were women. The ages varied from 34 (a patient with Hodgkin's disease) to 88, with an average of 70 years. Clinically, most cases were instances of ordinary cerebral apoplexy with more or less acute onset. Occlusive arteriosclerosis with or without microthrombosis was considered the underlying lesion. Nine patients were known to be hypertensive, one of whom was also diabetic. In three the ultimate cerebral symptoms developed as a complication of fracture or a surgical procedure.

In some cases the cerebral symptoms had started insidiously, but the duration varied from one day to about two weeks (in one case five weeks), averaging about nine days. The sites of the cerebral lesions varied, as in the preceding subgroup. At least one death (Case 81 with transdiaphragmatic perforating gastromalacia) was due to the peptic lesion, which was undiagnosed in all cases.

Types of Acute Peptic Lesions: The types encountered in the previously mentioned cases were esophagomalacia, involving the lower part of the esophagus, usually perforating into the left pleural cavity, 11 cases; perforating gastromalacia, involving the upper part of the stomach along the major curvature and posterior wall, 26 cases; multiple gastric erosion, 16 cases, and true acute gastric (pyloric) ulcer, 8

TABLE 3.—*Acute Cerebral Hemorrhage with Associated Acute Peptic Ulceration* *

Case No.	Sex	Age	Main Course; Survival Time; Intracranial Pathology	Esophagogastroduodenal Lesion (Death to Necropsy)	Group
189	M	47	Hypertensive; extensive hemorrhage in l. hemisphere; 5 hr.	Hemorrhagic duodenal mucosa and contents (21 hr.)	E
56	F	56	Hypertensive, sudden apoplexy; enormous hemorrhage r. hemisphere; 6 hr.	Gastromalacia	B
195	F	45	Diabetic, acute apoplexy; extensive hemorrhage in 1 hemisphere; 9 hr.	Hemorrhagic gastroduodenal mucosa and contents (15 hr.)	C E
32	F	44	Hypertensive, acute apoplexy and hematemesis; large r. cerebellar hemorrhage 10 hr.	Hemorrhagic duodenal erosions (12 hr.)	E
171	F	41	Epileptic; found dead with pill bottle; large arteriovenous hemangioma with hemorrhage in l. hemisphere; barbiturate in blood 18 mg/100 ml. (10 hr.); spontaneous hemorrhage + suicide (?)	Hemorrhagic gastric mucosa and contents (24 hr.)	C
179	M	54	Found unconscious; extensive hemorrhage l. hemisphere; 10 hr.	Large perf. gastromalacia with inflammatory reactions (15 hr.)	B
190	F	75	Hypertensive, sudden apoplexy; hemorrhage in l. int. capsl. & pons; 12 hr. (Fig. 2)	Perf. gastromalacia with inflammatory reactions (13 hr.)	B
43	M	55	Hypertensive, dysphasia; extensive acute hemorrhage l. hemisphere; 12 hr.	Gastromalacia (28 hr.)	B
92	F	71	Hypertensive, ac. apoplexy; extensive hemorrhage r. hemisphere; 16 hr.	Perf. gastromalacia and incipient esophagomalacia (24 hr.)	B A
208	F	62	Diabetic, hypertensive; large intraventricular hemorrhage; 17 hr.	Hemorrhagic gastric mucosa and contents (24 hr.)	C
192	M	46	Probably hypertensive, sudden apoplexy; hemorrhage in pons-mesencephalon; 24 hr.	Perf. esophagomal. with vital reactions (43 hr.). See Fig. 4 to 6 in previous paper [6]	A
193	F	60	Hypertensive, colitis; extensive hemorrhage r. hemisphere; 24 hr.	Gastromalacia and small subacute gastric ulcers	B (D)
41	M	23	Acute leukemia with multiple cerebral hemorrhage; about 1 day	Hemorrhagic gastric erosions (nonleukemic) (25 hr.)	C
378	M	60	Alcoholic addict; hypertensive; found dead; extensive hemorrhage r. hemisphere; 1 day (?)	Ac. duodenal ulcer (3 days)	E
307	M	68	Found dead; hemorrhage r. basal ganglia; 1 day (?)	Pyloric ulcers (erosions) (42 hr.)	C
27	M	51	Probably hypertensive; large ac. intraventricular hemorrhage from l. occip. lobe; 33 hr.	Two ac. pyloric erosions+gastro duod. petechiae (28 hr.)	D
6	M	56	Hypertensive; large hemorrhage l. occip. lobe; 43 hr.	Gastromalacia (25 hr.)	B
199	M	77	Peptic ulcer, diagn. 15 yr. previously; treated, med.; enormous hemorrhage r. hemisphere; 2 days	3 ac. pyloric ulcers (38 hr.)	D
40	M	54	Polycystic kidneys, hypertension; 2 days blurred vision, then pontine hemorrhage; 2 hr.	Gastroduod. erosions + chron. pyloric ulcer (40 hr.)	C D chr.
46	M	45	Polycystic kidneys, hypertension; acute hemorrhage l. basal ganglia; 3 days; ultimately hematemesis; see Fig. 1 in previous paper [6]	Esophagomalacia, 1 liter hemorrhagic gastric acid in l. pleura; gastromalacia; ac. pyloric ulcer; bloody stools (43 hr.)	A B D
1	M	34	Polycystic kidneys, hypertension; large hemorrhage r. occip. lobe (4 days)	Two 0.5-2 cm. ac. duodenal mucosal ulcerations (11 hr.)	E
183	M	60	Leukemia 4 mo.; 2 cm. hemorrhage in r. hemisphere days (?)	4 small ac. pyloric ulcers (22 hr.)	D
212	F	65	Hypertensive, apoplexy 4 yr. previously. Now extensive hemorrhage r. hemisphere, 5 days	Esophagomalacia	A
60	M	58	Diabetic; hemiparesis: recent hemorrhage and softening l. basal ganglia: 7 days	8 mm. pyloric ulcer	D
180	M	71	Hypertensive, sudden apoplexy; extensive hemorrhage r. hemisphere; 7 days	Gastromalacia (5 hr.)	B
170	M	63	Headache, unconsciousness and hemiparesis; death 8 days later; extensive l. subdural hematoma and minor hemorrhage in pons-mesencephalon: headache 2 wk., hemiparesis 1 wk.; probably traumatic, but no trauma known	Perforating transdiaphragmatic gastromalacia	B
52	M	33	Large hemorrhage l. mesencephal. and pons on left, gradual symptoms (10 days)	Small ac. duodenal ulcer (9 hr.)	E
88	F	71	Hypertensive; sudden hemiparesis; walnut-sized hemorrhage r. hemisphere; secondary pontine hemorrhage (2 wk.)	4 gastric erosions (35 hr.)	C
89	M	76	Gastroenterostomy previously performed for ulcer. Cerebral sympt. (3 wk.). Cortical-subcortical hemorrhages.	Old gastroenterostomy and jejunal peptic ulcer; nonperf. malacia of stomach and duodenum (44 hr.)	(B) E E chr.
219	M	65	Hypertensive; extensive hemorrhage l. hemisphere (3 wk.)	Hemorrhagic gastroduodenal mucosa and contents (19 hr.)	C

* Thirty cases listed as in Table 2.

TABLE 4.—*Acute Thrombo-Embolic Cerebral Infarction with Associated Acute Peptic Ulcer* *

Case No.	Sex	Age	Main Course; Survival Time; Intracranial Pathology	Esophagogastroduodenal Lesion (Death to Necropsy)	Group
82	M	59	Hypertensive, sudden hemiplegia; died 22 hr. later; thrombosed l. middle cereb. art.; incipient emollition	Perforating esophagomalacia with vital reactions (15 hr.)	A
79	M	53	Hypertensive; 2 yr. previously hemiparesis and hematemesis, now found unconscious; died 23 hr. later; thrombosis r. med. cereb. art., incipient emollition	Gastromalacia (18 hr.)	B
324	F	43	Hemiparesis due to thrombosis of r. sigmoid sinus following mandibular resection; survived 1 day	Perforating gastromalacia	B
21	M	47	Sudden r. hemiparesis following appendectomy; death 28 hr. later; thrombosis l. carotid and mid. cereb. art. with marked infarction	Perforating malacias of both esophagus and stomach (17 hr.)	A B
217	F	69	Treated for peptic ulcer at ages of 25 and 46; rheumatic heart disease; embolism both legs and r. int. carotid artery with extensive infarction r. hemisphere; death after 2 days	Duodenal erosions; no chronic ulcer or scar (24 hr.)	E
227	M	73	Hypertensive; sudden hemiplegia; death on 5th day; thrombosed l. mid. cereb. art. with extensive infarction l. hemisphere	Incipient esophagogastromalacia and 3 superficial gastric ulcers (32 hr.)	(A) (B) D
214	M	77	Hypertensive with old and recent cardiac infarctions; last day cramps and coma; embolus in l. post. cereb. artery with softening in occipital lobe and cerebral edema	Gastromalacia, perf. at necropsy (38 hr.)	B

* Seven cases listed as in Table 2.

cases. In 16 cases there were duodenal lesions. The total number of lesions surpassed the number of patients, as seven patients displayed two, and two displayed even three, types of lesions. In three cases chronic ulcers also were present.

II. Cases of Chronic Peptic Ulceration and Cerebral Vascular Lesions.—This main group of 33 patients (Table 6) includes 9 with chronic gastric and 2 with duodenal ulcer, 16 with ulcer scars, of which 12 scars were gastric and 4 duodenal (1 patient had both), and 7 with gastric resection performed for ulcer. Two had a gastroenterostomy. Three patients with concomitant acute ulceration were mentioned previously. Seventeen patients were men and 16 were women. The ages varied from 37 to 88 years, with an average of 71 years.

Subarachnoid hemorrhage was not seen in this group. Nine patients had recent major cerebral hemorrhage, while one pa-

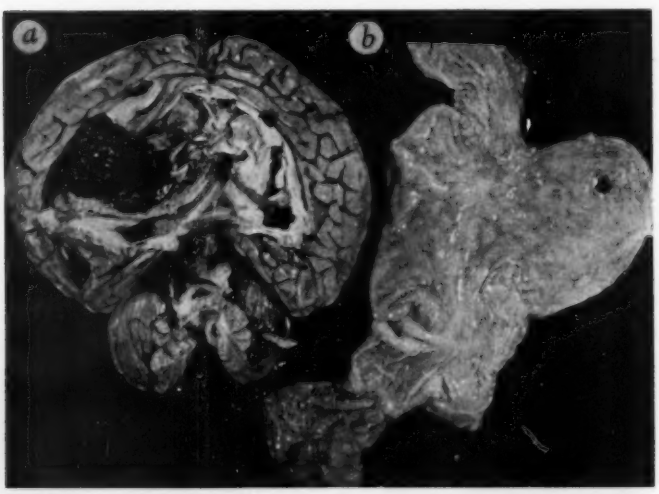

Fig. 2 (Case 190).—(*a*) Incomplete median section of the brain with large hemorrhage invading ventricles of a 75-year-old woman, who died within 12 hours after onset of symptoms. (*b*) Stomach displaying malacia with minor perforation. Sections from margin showed edema and cellular infiltration, indicating vital reactions.

Fig. 3 (recent case).—Acute gastric and duodenal ulcers found incidentally at necropsy in a 58-year-old hypertensive woman with rheumatic heart disease and cerebral embolism who died on the fourth day. Upper part of the gastrointestinal tract contained blood. Reproduced from the "Proceedings of the World Congress of Gastroenterology, 1958," by permission of the publishers, Williams & Wilkins Company, Baltimore, 1959.

tient with leukemia displayed multiple petechial cerebral hemorrhages. Two had recent and one had old cerebral thrombosis. In the remaining cases there were softenings similar to those of the preceding subgroup. The sites of the cerebral lesions varied considerably.

In some cases the information available was inconclusive for an estimation of the age of the cerebral or peptic lesions. Through comparison of case histories and necropsy findings, however, it appeared that in 9 of the 11 patients with recent cerebral lesions and in at least half of the remaining patients, the ulcer symptoms antedated the cerebral symptoms by one to several years. In some cases no ulcer symptoms had been mentioned. A few cases were intricate; that is, one patient (Case 23) also had a head injury, and another (Case 276) had an old psychosis, recent softening of the brain, and a subacute ulcer. One Case (No. 293) was seen by another pathologist, but no histologic sections were taken; it might be a case of acute ulcer. The same might be true of another patient (Case 152), who bled to death from her ulcer.

In the remaining cases it appears probable that the cerebral lesion antedated the peptic lesion; however, no proof of an etiologic relation could be obtained in any case.

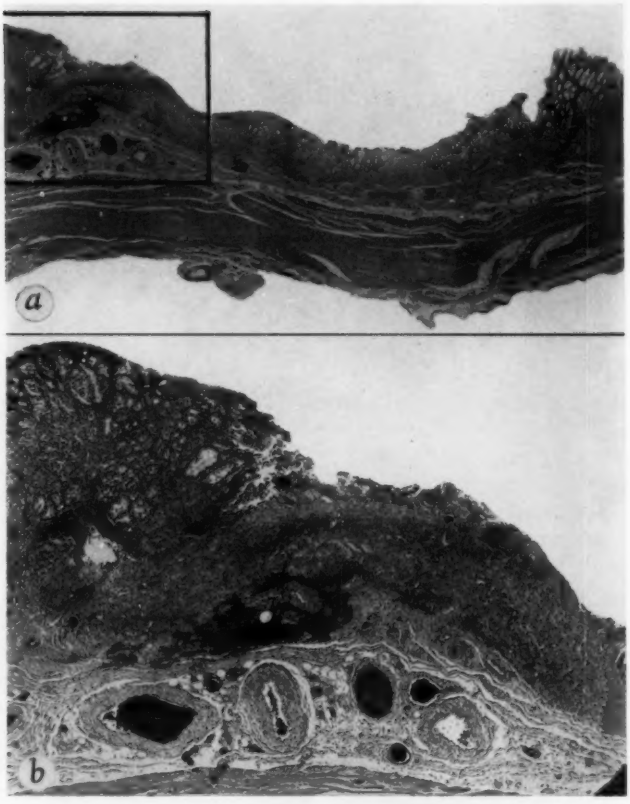

Fig. 4.—Same case as that represented in Figure 3. (*a*) Low-power view of the gastric ulcer. Hematoxylin and eosin; ×10. (*b*) Magnification of the framed corner of the ulcer shown in (*a*). Hematoxylin and eosin; ×30. Note recent hemorrhage and cellular infiltration, but complete absence of fibrosis and old vascular changes. The ulcer is acute.

Comment

In previous papers by others and by me a close association between certain types of cerebral lesions and acute peptic ulceration, including the so-called malacias, has been demonstrated. A similar association between cerebral lesions and chronic peptic ulcers was less convincing, although probable in some cases.

Concerning cerebral vascular lesions, Baló [1-3] found that these may induce acute, but probably not chronic, peptic ulcers. These results are confirmed by the material reported on here.

Except for three patients with concomitant chronic ulcers, the patients with *acute ulcerations* had no previous history of ulcer. They succumbed after massive cerebral apoplexy, which obviously antedated, and without doubt caused, the acute ulcerations incidentally found at necropsy.

Most of these patients were unconscious previous to death and displayed other severe primary symptoms, a fact which explains why the acute ulcerations were not diagnosed in any case. The interval from onset of the apoplexy to death, with the ulcer established or malacia initiated, was less than 12 hours in isolated cases, and in most cases the interval was only a few days. This is consistent with known facts concerning the rapidity with which acute neurogenic ulcers may occur. No predominant sites of the lesions in the brain, notably no predominance of diencephalic lesions, could be found in the cases of ulcer. This is in keeping with the findings of most recent, but not all, investigators on neurogenic ulcerations.

TABLE 5.—*Acute or Subacute Cerebral Softening with Associated Acute Peptic Ulceration* *

Case No.	Sex	Age	Main Course; Survival Time; Intracranial Pathology	Esophagogastroduodenal Lesion (Death to Necropsy)	Group
172	M	49	Dietetic treatment for ulcer, 25 yr. previously, hypertension; last yr. 3 apoplexies, last attack fatal in 24 hr., emollition in l. hemisphere and secondary hemorrhage in pons	Incipient gastromalacia with vital reactions; no evidence of chronic ulcer (24 hr.)	B
22	M	74	Hypertensive with acute r. hemiparesis; death in 5 days; large recent infarction l. hemisphere; also cardiac infarction	Three acute gastric erosions	C
81	M	71	Hypertensive; sudden cerebral insult, semicomatose, death in 5 days; pronounced cerebral atheromatosis but no visible thrombus	Gastromalacia with transdiaphragmatic perforation into l. pleural cavity, containing 1 L. of gastric acid	B
16	F	64	L. hemiparesis following apoplexy 10 yr. previously; death 5 days after operation for gallstone ileus; old and recent softenings in both basal ganglia	Esophagomalacia; gastric and duodenal erosions (16 hr.)	A C E
206	F	79	Hypertensive and diabetic, cerebral apoplexy with transient l. hemiparesis 12 & 3 yr. previously; admitted for fractured femoral neck; unconsciousness and death on 5th day; cerebral atheromatosis but no focal lesion found	Gastric erosion, 8 mm. in size, on minor curvature, 8 cm. above pylorus (16 hr.)	C
203	M	82	Arteriosclerotic with cardiac infarction; cerebral symptoms for the last 6 days, due to recent softening in external capsule	Acute duodenal ulcer, 5×5 cm. (4 hr.)	E
165	M	50	Hypertensive with acute l. hemiparesis, with coma and death on 6th day; recent necrosis r. basal ganglia and pons	Perforating esophagomalacia and (old ?) pyloric ulcer (9 hr.)	A D chr.
343	M	61	Apoplexy 15 yr. previously; now admitted after fall; cardiac and cerebral symptoms; death on 6th day; incipient softening r. hemisphere	Small pyloric ulcer (no microscopy) (44 hr.)	D
25	M	73	Admitted for prostatic hyperplasia with urinary obstruction; hypertensive; developed atrial fibrillation and ultimately unconsciousness; small recent emollition r. occipital lobe	Perforating gastromalacia and acute hemorrhagic gastric erosions (50 hr.)	B C
28	M	34	Hodgkin's disease with terminal generalization; last days comatose; encephalomalacia r. basal ganglia	Perforating gastromalacia + acute duodenal ulcer	B E
228	F	69	Fractured femoral neck; nailing followed by cerebral impairment and r. hemiparesis; death on 11th day; multiple recent cerebral softenings	2 acute duodenal ulcers (10 hr.)	E
11	M	76	Large infarction r. hemisphere; no visible thrombus (Fig. 5)	2 acute prepyloric ulcers (19 hr.)	D
158	F	88	Acute apoplexy with l. hemiparesis and coma; death in 15 days; acute hemorrhagic infarction r. frontoparietal lobe	Acute hemorrhagic duodenal erosions (5 hr.)	E
76	F	68	Progressive cerebral insufficiency with disablement following apoplexy 5 yr. previously; ultimately cramps and fever; pronounced cerebral atrophy mainly of frontal lobes (also cranial hyperostosis)	Perforating gastromalacia	B
201	M	71	Hypertensive; transient hemiparesis 7 and 4 yr. previously; now sudden hematemesis and r. hemiparesis; death in 3 wk.; cerebral arteriosclerosis with minor softenings in both basal ganglia	Hemorrhagic gastric mucosa and contents (24 hr.)	C
222	F	78	Hypertensive; one yr. previously apoplexy with hemiparesis; died in cardiac insufficiency; subacute softening 4×1 cm. in r. hemisphere	Gastromalacia with vital reactions; perforated at necropsy (33 hr.)	B
17	M	67	Hypertensive with l. hemiplegia 9 mo. and again 5 wk. ante mortem; softening r. motor region	Gastromalacia (15 hr.)	B

* Seventeen cases without major hemorrhage or gross thromboembolism.

The patients with subarachnoid hemorrhage were generally younger; those with cerebral hemorrhage were of intermediate age, and those with arteriosclerotic softening were mainly elderly patients. These variations conform with those of cerebral vascular lesions in general. As found in other categories of patients with neurogenic ulcers, those with acute ulcerations were generally younger than those with chronic ulcers.

In some cases of *chronic peptic ulcer* evaluation was difficult because the cerebral lesions were small, and information per-

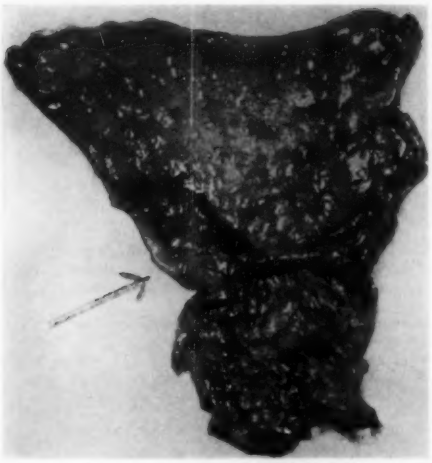

Fig. 5 (Case 11).—Two acute prepyloric ulcers from a 76-year-old man who had acute hemiplegia due to extensive infarction in the right hemisphere and who died on the 15th day after infarction. He gave no history of ulcer.

mitting a dating of the cerebral and peptic symptoms was lacking. Analysis of all information available, however, revealed that in most cases the ulcer symptoms had antedated the cerebral symptoms. This does not exclude a positive correlation but rules out the cerebral lesion as the immediate cause of the ulceration. In the remaining cases of chronic ulceration an etiologic relation to the associated cerebral lesion likewise could not be proved.

Because of the high incidence of both cerebral vascular lesions and peptic ulcers in general, the possibility of coincidence by mere chance must be considered. A statistical analysis might solve this question. However, the basic material is hardly fitted for such analysis, as it includes different hospital and medicolegal necropsy series, not directly comparable, as reflected, for example, in varying age and sex distributions. Furthermore, during the first part of the study the brains were not examined routinely unless evidence of an intracranial lesion was at hand, and some minor cerebral lesions might have escaped notice.

A few basic figures might, nevertheless, be considered. The proportion of cerebral vascular lesions of the types here concerned in the basic material was about 12%, estimated from the (main) part of the material which permitted such evaluation. Of the 208 patients with acute peptic ulceration, however, 32% had cerebral vascular lesions, a figure which obviously suggests a correlation. Of the 167 patients with chronic ulcers, 18.6% had cerebral vascular lesions. Apart from the difference between the percentages of acute and chronic ulcer cases, the former were generally associated with more acute and severer cerebral lesions than were at least some of the chronic ulcer cases.

The cases of cerebral vascular lesions here considered seemed to be ordinary instances thereof. Why just these cases were complicated by ulcers is not exactly known. Baló has considered some points, mentioned in the introduction. A few ulcers appeared to be already in a process of healing, and it is reasonable to assume, from analogy with other neurogenic ulcerations, that the acute ulcers might have healed if the patients had survived the primary cerebral lesion. This does not apply to the cases of malacia.

Summary and Conclusions

Cerebral vascular lesions are rarely mentioned among the causes of neurogenic peptic ulceration. In the present study, however, 32% of 208 patients with acute peptic ulceration, including esophageal and gastric malacias, had such lesions. Cerebral vascular lesions were present in 18.6% of 177 necropsy cases displaying chronic ulcers, including sequelae.

Clinical and pathologic evidence concerning the relationship between the cerebral and the peptic lesions is considered. Contrary to what was observed in the cases of acute ulceration, the dyspeptic symptoms in the cases of chronic ulcer usually antedated the terminal cerebral symptoms, and, although a correlation appeared possible, an etiologic relationship could not be proved in these cases.

Concerning the acute ulcerations, however, the observations suggest an etiologic relationship, supporting the following con-

TABLE 6.—*Vascular Cerebral Lesion Associated with Chronic Peptic Ulceration* *

Case	Age	Sex	Duration of: Cerebr. Sympt.	Duration of: Ulcer History	Necropsy Findings: Cerebral Lesion	Necropsy Findings: Lesion of Gastrointestinal Tract	Hours Post Mortem
144	82	F	10 hr.	No	Large hemorrhage	Scar lesser curvat.	15
293	37	M	1 day	No	L. parietal hemorrhage	Small gast. ulcer (acute ?) †	24
368	55	M	1 day	?	Large hemorrhage	Duodenal scar	28
269	67	F	1 day	1 yr.	Softening r. internal capsule	Ulcer lesser curvat.	46
272	60	F	1 day	33 yr.	Large hemorrhage	Scar lesser curvat.	25
366	55	M	2 days	10 yr.	Large l. frontal hemorrhage	Pyloric scar	34
232	48	F	3 days	?	Thrombosed l. post. cereb. art.	Stomach resected (at 42)	34
276	65	F	Days	No	Frontal atrophy & softn.‡	Subchronic gastric ulcer	40
277	78	F	Days	No	Softn. l. internal capsule	Scar lesser curvat.	8
137	71	M	Days	4 yr.	Minor softn. basal ganglia	Large duodenal ulcer §	29
282	56	F	Days	10 yr.	Petechial hemorrhages ‖	Duodenal scar	11
152	77	F	1 wk.	1 wk.	Small l. cerebellar infarct.	Two pyloric ulcers (acute ?) §	?
105	80	M	8 days	40 yr.	Softening brain stem	Pyloric scar; gastroent. (at 79)	21
235	77	M	10 days	6 yr.	Cereb. atrophy, cerebellar softn.	Scar lesser curvat.	17
304	77	F	10 days	19 yr.	Large hemorrhage	Stomach resected (at 61)	18
267	58	M	19 days	20 yr.	Small softening r. hemisphere	Stomach resected (at 38)	11
117	87	F	23 days	No	R. carotid thrombosed	Duodenal ulcer	23
273	72	M	1 mo.	4 yr.	Large softn. r. hemisphere	Ulcer lesser curvat.	18
145	62	M	3 mo.	3 yr.	Large hemorrhage	Small pyloric ulcer	21
238	72	M	3 mo.	7 yr.	Softenings l. basal ganglia	Scar lesser curvat.	7
104	69	F	3 mo.	9 yr.	Softening r. hemisphere	Stomach resect. (at 60)	30
116	86	F	Months	No	Softening r. basal ganglia	Large ulcer lesser curvat.	65
263	69	F	8 mo.	10 yr.	Multiple softenings	Pyloric scar	40
153	75	M	1 yr.	6 yr.	Softn. r. basal gangl. & pons	Two pyloric ulcers §	22
264	69	M	14 mo.	No	Large softening l. hemisphere	Scar lesser curvat.	12
133	49	M	15 mo.	23 yr.	Large hemorrhage	Gastroduod. scars, gastroent. (at 26)	32
283	74	F	16 mo.	No	Thrombotic atrophy	Scar lesser curvat.	13
110	69	F	2 yr.	30 yr.	Questionable case ¶	Stomach resected (at 41)	?
250	88	M	Years	No	Large softn. l. hemisphere	Pyloric scar	19
300	64	M	6 yr.	20 yr.	R. temporal cystic softn.	Stomach resected (at 45)	19
122	77	F	15 yr.	No	R. frontal hemorrhage	Duodenal scar. Diabetic	11
240	77	M	19 yr.	13 yr.	R. parieto-occip. softn.//	Large gastric ulcer	6
230	49	M	34 yr.	22 yr.	Atrophy and softenings	Stomach resected (at 45)	8

* Thirty-three cases with chronic gastroduodenal ulcers, ulcer scars, or resection performed for ulcer.
† Examined by another pathologist, who considered the ulcer to be a small chronic one.
‡ Schizophrenic for 30 years; acute cerebral symptoms a few days.
§ Died of large gastrointestinal hemorrhage.
‖ Died of acute leukemia.
¶ Clinical evidence of cerebral vascular lesion, epilepsy, also uremia. Necropsy revealed only slight atrophy of brain.
// Also neurosyphilis.

clusions: Cerebral vascular lesions are the commonest single cause of acute peptic ulceration, as encountered at necropsy. Such acute neurogenic lesions may occur within 12 hours, but are found mostly a few days after the apoplexy. They usually escape clinical diagnosis, even in cases in which the complicating ulceration is the immediate or contributing cause of death.

Cerebral vascular lesions should be considered frequent and clinically important causes of neurogenic peptic ulceration.

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Human Filarial Infection in Louisiana

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In the United States cases of subcutaneous nodules in humans caused by microfilariae have been reported in Florida. To our knowledge the present case represents the first such occurring in Louisiana. Limitation of this condition to the southeastern states suggests that the causative agent is restricted to this area, but the geographic distribution of reported cases may be due in part to chance alone. It seems advisable that close scrutiny of subcutaneous inflammatory nodules with examination of multiple sections be done on such surgical specimens received in other parts of the country to determine whether or not human filarial infection may be more widespread in the United States than it now seems to be.

Report of a Case

In November, 1957, a 28-year-old white man from Kentwood, La., was admitted to the Illinois Central Hospital in New Orleans with a complaint of soreness and swelling in the right arm. His illness had started three weeks previously with the appearance of a painful nodule in the medial side of his right arm just above the elbow and red streaks running up the arm. He was treated with penicillin and bed rest at that time, and the symptoms disappeared within a week. When he returned to manual labor, although he was still taking oral penicillin, his forearm became red, swollen, and warm, and he was thought by his local surgeon to have erysipelas. The swelling and redness subsided in a few days, when the forearm was rested and soaked in hot magnesium sulfate U.S.P. (epsom salt) solution several times a day, but a small tender nodule remained palpable under the skin of the extensor surface of the forearm about three inches below the elbow. Covered with fiery-red skin, the entire lesion superficially resembled a boil. When the patient returned to duty, the swelling of the forearm returned, but it subsided again with rest.

The past medical history and system review were noncontributory. The patient had lived in Kentwood, La., almost his entire life. With the exception of two years spent in San Antonio, Texas, while in the Army, and short visits to Mexico City and Chicago, he had not been outside southern Louisiana or the neighboring portion of Mississippi. He was fond of hunting, especially raccoons, and had had several hounds and coon dogs, later found to have Dirofilariasis. He had three dogs at the time of his illness.

Physical examination gave completely normal findings except for the presence of a subcutaneous nodule palpable under the skin of the extensor surface of the right forearm about three inches below the elbow.

A clinical diagnosis of irritative foreign body was made, and he was admitted for its removal. Routine laboratory studies, including urinalysis, complete blood count, and a blood Wassermann test, provided normal results. There were 5,250 white cells per cubic millimeter of peripheral blood, and the differential blood count was neutrophils, 26%; lymphocytes, 68%; monocytes, 1%; eosinophils, 4%, and basophils, 1%. X-ray of the forearm was negative.

The nodule was removed by dissection, under local anesthesia, fixed in 10% formalin, and submitted to one of us (F. H. H.) for examination.

Pathology

Gross.—The specimen measured 2.5×2×1.3 cm. and consisted mostly of soft, yellow fat. One zone, occupying about the central third of the specimen, and extending more toward one surface, was somewhat firmer than the rest of the specimen, and on sectioning showed a somewhat cream-colored surface. Three sections through this area, including the soft, yellow fat attached, were submitted for microscopic study.

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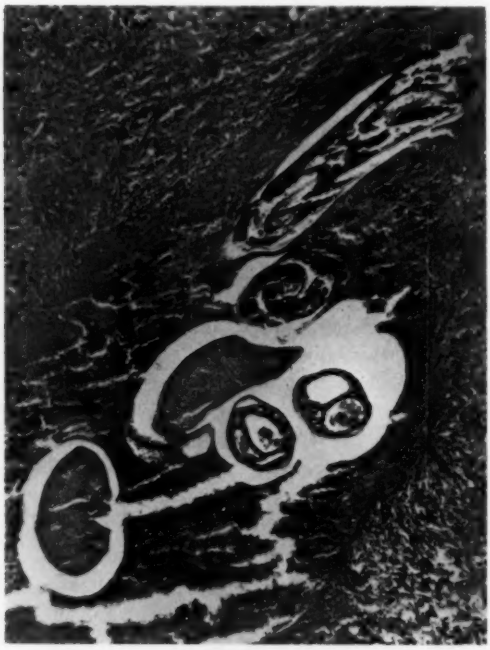

Section through granuloma, showing portions of filarial worm.

Microscopic (Figure).—Sections showed portions of normal fibrofatty tissue, with a central area which was intensely infiltrated with lymphocytes and neutrophils. There was marked vascular and fibroblastic proliferation in this area. In two different regions within the infiltrated area there were irregular spaces which contained sections of structures having the morphological characteristics of portions of a worm. Tangential, longitudinal, and transverse sections of the worm were visible. Surrounding these structures there was necrotic material, containing innumerable neutrophils, many of which were fragmented and had pyknotic nuclei. Immediately adjacent to this, surrounding the area, there were reticuloendothelial cells, some of them epithelioid in appearance and accompanied by a few multinucleated giant cells.

Microscopic Diagnosis.—Fibrofatty tissue containing granulomatous inflammatory reaction to structures suggestive of portions of a worm.

The sections were referred for identification to Dr. Paul Beaver, who found them to be compatible with a degenerating adolescent female Dirofilaria sp., similar to those called Dirofilaria conjunctivae.

Although, unfortunately, no serum was taken for immunologic studies before biopsy, about one month afterward serum from the patient was tested for the presence of antibodies to Dirofilaria by means of the hemagglutination and complement-fixation tests. Both gave negative results. Blood from the patient's dogs contained microfilariae of Dipetalonema, but his own blood contained no parasites.

Comment

Thirty-nine instances of human infection with Dirofilaria have been reported (Faust, 1957; Sams and Beck, 1959). While distribution of these reports has been widespread, of the six previous reports in the United States, five have been from Florida (Faust et al., 1952; Faust, 1957; Sams and Beck, 1959) and one from New Orleans (Faust et al., 1941). The Florida cases were all of superficial infection, such as the present one, but the New Orleans case

was of adult Dirofilaria infection of the vena cava. Most of the other superficial infections, like this one, have been with immature female worms. Death of the worm before sexual maturity is probably an evidence of the unsuitability of the human host.

In the present case there was a history of a nodule above the elbow early in the illness, before the appearance of the nodule in the forearm. While this might have represented the wandering of the parasite, as in one of Faust's (Faust, 1957), and one of Sams and Beck's cases, the lesion was more likely epitrochlear lymphadenitis, since the patient's illness probably began with the death of the worm.

It is interesting to speculate on the origin of the infection. In the Mediterranean region and the Near East, human superficial Dirofilarial infection may be with D. repens, which is a common subcutaneous parasite of dogs in that region, but this species has not been identified in the United States. It is possible that this man's infection was with Dirofilaria immitis, the common heart worm of dogs, since this species, even in its normal host, resides for a time in the subcutaneous tissues before moving to the heart (Kume and Itayaki, 1955). On the other hand, another Dirofilaria species cannot be ruled out in this instance and may be, in fact, a more probable suspect. D. tenuis (Chandler, 1942), a subcutaneous parasite, very similar to D. repens, is found in a large percentage of the raccoons in southern Louisiana (Orihel, 1959), and these animals abounded in the woods about this patient's home. Other species of Dirofilaria, namely scapiceps (of rabbits) and spinosa and subcutanea (of porcupines) have been reported from the United States. While rabbits occur in the patient's district, porcupines do not.

The possibility of infection with Dipetalonema sp. in this patient was considered in view of the presence of microfilariae of that species in the peripheral blood of his dogs. To test this hypothesis, the sections were measured and the maximum diameter found to be 117μ, as compared with a diameter of 140μ for Dipetalonema sp. from local dogs. Thus, infection with Dipetalonema cannot be excluded on this basis.

Our uncertainty in this matter illustrates the need for further studies on the microanatomy of the commoner filariae and other types of worms. The articles of Nichols (1956) and that of Orihel (1959) have shown that this is feasible to a degree not heretofore thought possible.

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Histochemical Variations with Age in Roosters' Testes

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The process of aging must involve changes in the biochemical character of tissues, as well as commonly observed morphologic changes. These biochemical changes can be evaluated with histochemical techniques. Previous works on histochemical changes with age have been reported on the adrenal cortex,[1] costal cartilage,[2] aorta,[3] ureters,[4] senile plaques in the brain,[5] and other tissues.[6,7] The testis is an especially good tissue to study for histochemical differences with age, as hypospermatogenesis provides a morphologic criterion for comparison. In addition, it has proved feasible to tissue-culture testicular germ cells. We have studied the testes of various-aged roosters and tissue cultures of these testes in a search for changes with age.

Materials and Methods

Testes were obtained from about 100 chicken embryos, about 12 adult Rhode Island Red and White Leghorn roosters 6 months to 2 years of age, and from 6 older roosters, 3 to 6 years of age. The older roosters were a New Hampshire Red 5 to 6 years of age, a White Rock 5 to 6 years of age, a cross-breed White Leghorn and game rooster 4 to 5 years of age, a cross-breed Plymouth Rock-White Rock 4 to 4½ years of age, and two game roosters 3 years of age. Histochemical studies on tissues were made immediately after removal. Testes were cultured for three to seven days, the length of time depending upon the technique employed and the time required to get adequate new growth for testing. Two different methods were used for tissue cultures. The first was to culture cells as radial new growth from an explant approximately 1 mm. in diameter implanted upon a plasma clot. The other method was a seeding technique, in which pieces of roosters' testes were immersed in culture fluid and fragmented with Bard-Parker knives into 50μ to 500μ particles. A suspension of the particles in 0.85% saline was then discharged into Porter flasks containing coverslips, and the particles were allowed to settle. The flasks were not disturbed for 48 hours. Culture medium consisted of 2 parts Hanks' balanced salt solution, 2 parts horse serum, and 1 part chicken embryo juice EE50. Cultures were incubated at 38 C.

Alkaline phosphatase was demonstrated by Pearse's modified coupling azo-dye method,[8] with changes as outlined by Burton.[10] Esterase was stained as suggested by Gomori.[11] Ribonucleic acid was demonstrated by the methyl green-pyronin stain, and deoxyribonucleic acid, by the Feulgen reaction. Lillie's[12] outline was followed for the periodic acid-Schiff reaction, the Sudan IV (scarlet red) stain for fat, and the Sudan black stain for the demonstration of formalin-fixed fat. The Sudan black reaction was performed on tissues after formalin fixation and processing through alcohol, and they probably demonstrate lipoprotein or phospholipid.

Results and Comment

The usual histochemical norms of adult rooster testes are listed in Table 1. These are the reactions characteristically found in young and middle-aged adult tissue. The representative reactions are portrayed in the Figures. For comparative results of similar studies on embryo tissues, the reader is referred to our previous publication.[13]

There were no differences in the morphologic or histochemical characteristics of tissue-culture cells, whether the culture was from embryo or young or old adult rooster testes, except that the growth of germ cells was greater and faster in cultures made from embryo testes. Histochemical reactions were of the same value in the identification of cells in tissue cultures of adult rooster testes as they were in identifying embryo germ cells in culture.[13,14] For instance, the tissue spermatogonia and primi-

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TABLE 1.—*Histochemical Reactions of Testes and Tissue Cultures of Testes of Adult Roosters*

Testes Tissue Sections	Alk. Phos.	Acid Phos.	Esterase	RNA	DNA	Sudan IV	Sudan Black	PAS
Germ cells (undifferentiated spermatogonia)	—	—	+ to +++C	+++C	+++N	+C	++C	++C
Germ cells (differentiated spermatocytes, sperm)	—	+++C	+C	+++C	+++N	+C	++C	++C
Sertoli cells	—C	+C	+++C	+C	+++N	++C	++C	
Basement membrane	++	—	—	—	—	—		+++
Basement-membrane cells	++C	—	—	+C	+++N	+C		+++C
Leydig cells	—	—	+	+C	+++N	0 to +++C	++C	
Indifferent interstitial area cells	+C	—	—	+C	+++N	—	—	
Capsule cells			—	+C	+++N		—	
Endothelial cells			—	+C	+++N	++C	+C	+C
Testes Culture Seeded								
Broad fusiform new-growth cells	—	+C	++C	+C	+N	0 to +++C	+++C	++C
Round germ cells near seeded culture	—	+++C	+++C	++C	++N	0 to +++C	+++C	+++C
Mass of seeded tissue	++	++	+++	+++	++N	+++	+++	+++
Testes Culture with Explant								
Broad fusiform new-growth cells	—	+C	+C	+C	+N	0 to +++C	+++C	++C
Round germ cells near seeded tissue	—	++C	++C	++C	++N	0 to +++C	+++C	++C
Mass of explant tissue	++	++	+++	+++C	++N	+++	+++	+++
Narrow fusiform cells	+C	—		+C	++N	0 to ++C	++C	++C

C=reaction in cytoplasm. N=reaction in nucleus.

tive, undifferentiated germ cells contained an abundance of cytoplasmic esterase, and this was also characteristic of germ cells in culture, aiding in the distinction of germ cells in both tissues and tissue culture.

There was no detectable difference in morphology or histochemical character among tissues or tissue cultures of testes of birds of different species. This failure to find species differences was true whether the testes was of an embryo or of an adult rooster.

Many of the histochemical tests were the same in the testes of roosters regardless of age except for minor differences. The Sertoli cells of the adult testes have some Sudan IV-positive granules in their cytoplasm, whereas there is no such material in the testicular tubules of the embryo. The basement membrane cells, endothelial cells, and Leydig cells in tissue contain fat droplets, and almost any cell in tissue culture may contain Sudan IV-positive material. Similarly, there is no difference dependent upon the age of the rooster in the ribonucleic acid and deoxyribonucleic acid and periodic acid-Schiff reactions in tissue or tissue culture of testes. There is Schiff-positive material in germ cells, basement membrane, basement membrane cells, endo-

TABLE 2.—*Changes with Age in Three Histochemical Reactions*

Testes Tissue	Acid Phosphatase	Esterase	Sudan Black
Norm for embryo			
Primitive germ cells	+	+	+
Primitive Sertoli cells	+++	+++	+++
Norm for adult roosters			
Spermatogonia	—	+ to +++	++
Spermatocytes and paraluminal material	+++	+	++
Sertoli cells ("ribs")	+	+++	++
Aged rooster reaction—			
Cross-breed White Leghorn and game rooster, 4 to 5 yr. old			
Spermatogonia	—	—	—
Spermatocytes and paraluminal material	+++	+++	+++
Sertoli cells ("ribs")	—	+	—

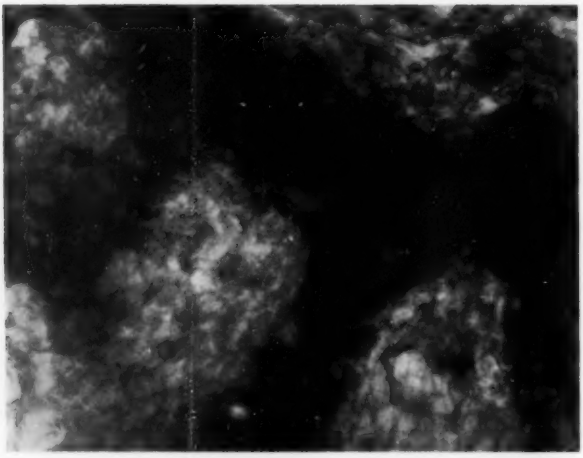

Fig. 1.—Acid phosphatase. Testis of 17-day chick embryo. Germ cell (double arrows) has a few granules positive for acid phosphatase, or a slight darkness of cytoplasm, whereas the Sertoli-cell precursor (single arrow) has dark cytoplasm, indicating much acid phosphatase. × 600.

thelial cells, undiffentiated tubule cells (embryo), and white blood cells.

Significant differences, dependent upon the age of the rooster, were found in acid phosphatase, esterase, and Sudan black reactions. In acid phosphatase reactions of embryo tissues, the more primitive germ cells, or spermatogonia, are slightly positive, while the early Sertoli cells (peritoneal cells of Swift)[15] are highly positive (Fig. 1). In adult tissues of both young cockerels and aged roosters, the Sertoli-cell "ribs" are positive for acid phosphatase, and the spermatocytes are quite positive, whereas the primary germ cells, or spermatogonia, contain almost no acid phosphatase (Fig. 2). Similarly, the acid phosphatase reaction has been depicted[16] in the adult rat as positive in Leydig cells, tubule basement membrane, and, possibly, intratubular supportive cells and more differentiated germ cells. While the Sertoli-cell areas ("ribs") have acid phosphatase in most of the adult rooster testes, in one 5-year-old cross-breed White Leghorn-game rooster, the acid phosphatase was found strictly in the paraluminal spermatocytes and not in Sertoli cells (Fig. 3).

The tissue esterase reactions of the embryo undifferentiated germ cells and spermatogonia are slightly positive, while the

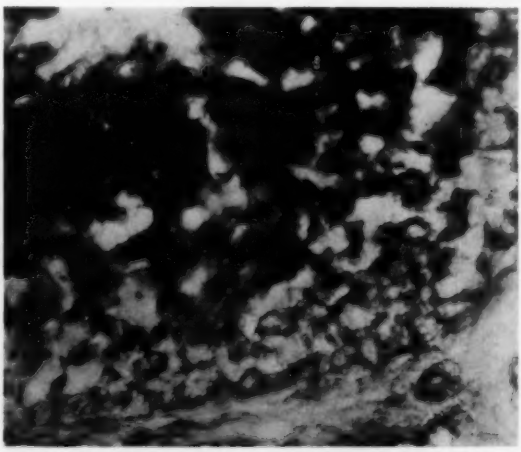

Fig. 2.—Acid phosphatase. Testis of one-year-old rooster. Note absence of any positive (dark) material in primitive germ cells along the tubule basement membrane, but its presence in the paraluminal, or spermatocyte, layer and slight tendency to formation of "ribs" in the central portion only. × 700.

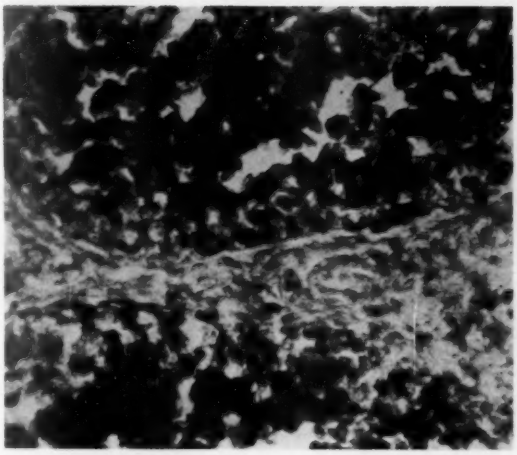

Fig. 3.—Acid phosphatase. Testis of a 4- to 5-year-old cross-breed White Leghorn and game rooster. As compared with Figure 2, there is more acid phosphatase in the primitive cells near the tubule basement membrane and no tendency to the outlining of "ribs" (Sertoli-cell areas). × 610.

Fig. 4.—Nonspecific esterase. Testis of 16-day embryo. Germ cell (double arrows) also has a few granules of esterase activity, whereas Sertoli-cell precursor (single arrow) is dark, with much esterase. × 800.

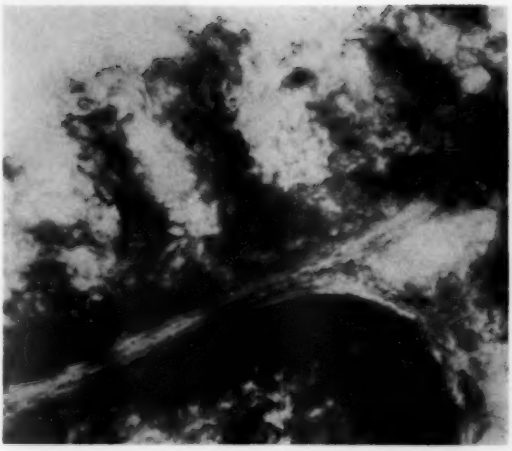

Fig. 5.—Nonspecific esterase. Testis of one-year-old rooster. Note abundant esterase (dark cytoplasm) in cells along basement membrane and in rib-like areas extending out from the basement membrane, representing primary germ-cell and Sertoli-cell areas. × 610.

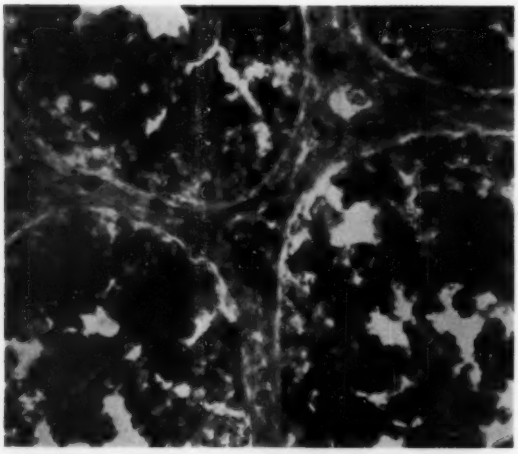

Fig. 6.—Nonspecific esterase. Testis of a 4- to 5-year-old cross-breed White Leghorn and game rooster. Note positive reaction of luminal contents and matured spermatocytes, but absence of reaction in spermatogonia or sustentacular cell portions. Compare with Figure 4. × 610.

peritoneal cells are highly positive (Fig. 4). Some of the adult tissue spermatogonia have very positive esterase reactions, and others are slightly positive. The spermatocytes have little esterase (Fig. 5). The Sertoli cells, in their repetitive rib-like zones at right angles to the basement membrane of the adult seminiferous tubules, are esterase-positive. Again, the 5-year-old cross-breed White Leghorn-game rooster exhibited an esterase reaction different from the adult norm and almost identical with adult acid phosphatase reaction, showing no reaction in spermatogonia or Sertoli-cell "ribs" but a pronounced paraluminal or spermatocyte reaction (Fig. 6). The demonstration of "ribs" in any preparation usually is associated with the formation of sperm. Comparatively, Pearse [8] depicts esterase in Leydig cells and some adjacent tubular basement membranes, but not in the germ cells of adult rats, whereas Nachlas and Seligman [17] found that not only the interstitial cells but the tubule germ cells of the adult rat contained esterase. Nachlas and Seligman also report apparently more esterase activity in the spermatogonia than in more mature germ cells of an adult dog.

The Sudan black reactions for formalin-fixed fat are also the same in both the adult

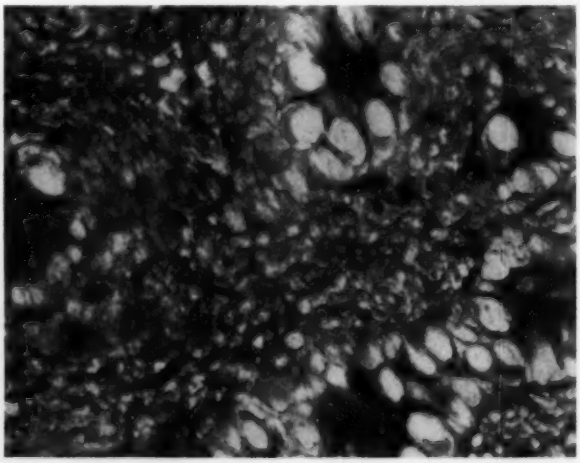

Fig. 7.—Sudan black. Testis of 16-day embryo. Note dark (Sudan black-positive) material in central portions of germinal cords and in crescent around nucleus of germ cells (arrows). × 690.

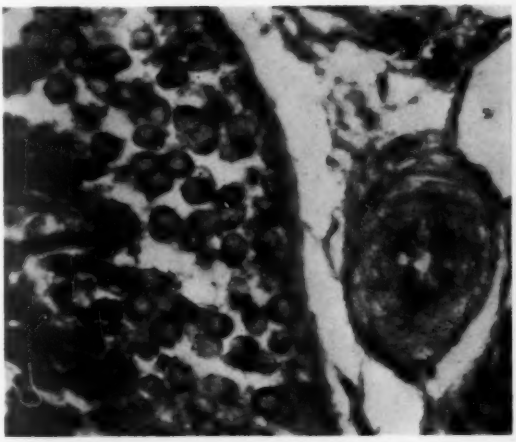

Fig. 8.—Sudan black. Testis of 2-year-old rooster. Note positive material in cytoplasm around nuclei of germ cells, within lumen, in interstitial cell areas, and in endothelium of blood vessels. × 630.

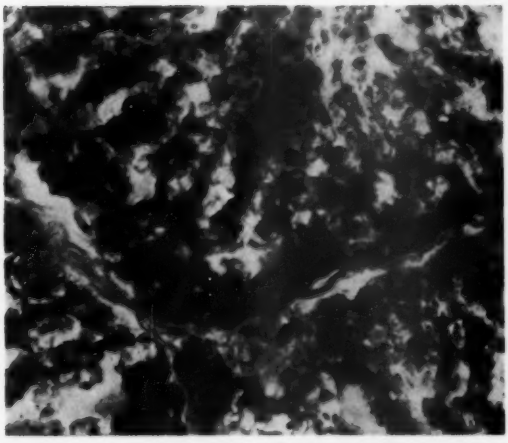

Fig. 9.—Sudan black. Testis of a 5- to 6-year-old New Hampshire Red rooster. Note predominance of reaction in spermatogonia or peripheral portion of tubule and in the sustentacular-cell zone ("ribs"). Compare with Figure 7. × 610.

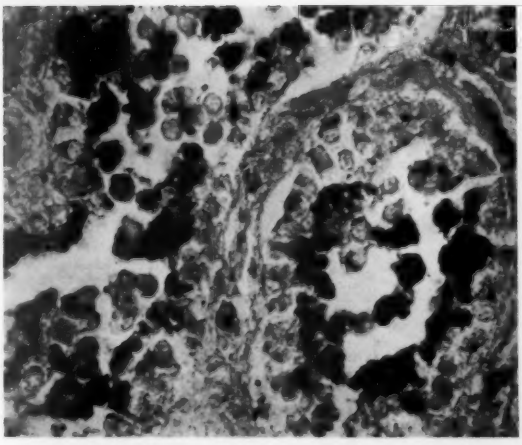

Fig. 10.—Sudan black. Testis of a 4- to 5-year-old cross-breed White Leghorn and game rooster. This reaction is similar to that of the 2-year-old cockerel (Fig. 8), but has more pronounced concentration of Sudan black-positive material in the paraluminal cells. Note lack of sperm. × 610.

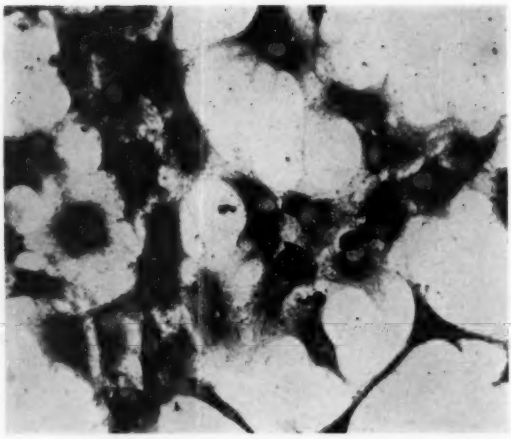

Fig. 11.—Sudan black. Tissue culture of testis of 2-year-old rooster. Most cells are germ cells. Note much positive material around nuclei in cytoplasm, as it occurs around the nuclei of germ cells in Figure 6. × 300.

and the embryo. The Sudan black reactions are of particular interest in that positive material is demonstrated in perivascular areas and in or around mitochondria in germ cells of both embryo and adult tissues (Figs. 7 and 8). The nuclear caps of mitochondria are particularly well outlined in the germ cells of tissue cultures, as well as tissues (Fig. 11). In the adult norm, the spermatogonia and paraluminal spermatocyte areas are both positive, as are the Sertoli-cell "ribs" (Fig. 9). Here, again, our aged rooster demonstrated an atypical reaction. The Sudan black reaction of the 4- to 5-year cross-breed rooster exhibited only paracentral deposition and the spermatogonia paranuclear cap reactions were less (Fig. 10).

These atypical acid phosphatase, esterase, and Sudan black reactions in the 5-year-old cross-breed rooster are associated with aspermatogenesis, morphologic evidence of degeneration that is found in advanced age.

Summary and Conclusions

There are consistent and reproducible norms of tissue alkaline and acid phosphatase, esterase, deoxyribonucleic acid, ribonucleic acid, periodic acid-Schiff, Sudan IV, and Sudan black histochemical reactions in embryo and young adult roosters.

There is no histochemical difference between roosters' testes due to species differences alone.

Tissue cultures of germ cells of all age groups of roosters have similar morphologic characteristics and similar histochemical reactions.

The most primitive cells of adult rooster testes differ from the most primitive germ cells of young chick or chick embryo testes by having little or no acid phosphatase, as compared with a moderate amount of acid phosphatase in the primitive germ cells of the embryo testes, and by having much nonspecific esterase as compared with relatively little esterase in the primitive germ cells of the embyro testes.

Roosters 3 years of age or older tend to have the same type of histochemical reactions as younger roosters, but to a less pronounced degree.

One 5-year-old rooster that had morphologic evidence of aging change in its testes also had acid phosphatase, esterase, and Sudan black reactions that differed from those of younger roosters.

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Antagonistic Effects of Parathyroid Extract and Cortisone

Effects on Serum Protein and Glycoprotein Fractions and on Renal Calcification

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Introduction

Cortisone has been shown to reverse the effects of parathyroid extract (PTE) on serum calcium, bone, and renal calcification.[1] The effects of PTE resulting in renal calcification and elevation of serum glycoprotein have been described previously.[2,3] Effects of cortisone on the serum total glycoprotein of rats have also been described.[4] The serum glycoprotein-elevating capacity of turpentine has been described and compared with that of PTE.[3]

The present investigation was undertaken to study the effects of simultaneous administration of cortisone and PTE on serum total glycoprotein, on the electrophoretic distribution of the serum protein and glycoprotein, and on the processes associated with calcification in the kidney. A parallel study of the effects of cortisone on the elevated serum glycoprotein levels induced by subcutaneous injection of turpentine was also undertaken.

Materials and Methods

EXPERIMENT A.—Twenty-four Holtzman male rats, each weighing 240 to 280 gm. (approximately 9 weeks of age), were divided into four groups of six each. Animals in Group I served as controls, receiving no treatment. Rats in Group II were given subcutaneous injections of 5 mg. of cortisone acetate twice daily for three days; those in Group III received subcutaneous injections of 0.45 ml. (45 units) of PTE twice daily for three days. Group IV rats received each of these two drugs at the doses indicated above. Seventy-two hours after the first injection each animal was anesthetized with ether, the peritoneal cavity opened, and blood drawn immediately from the abdominal aorta. The kidneys were then removed, sliced longitudinally into two pieces, and placed in 10% buffered formalin.

Histological sections of the kidney specimens were studied after preparation with the following stains: hematoxylin and eosin, Kóssa's stain for calcification,[5] periodic acid-Schiff (PAS) for neutral polysaccharides,[6] and Alcian blue for acid polysaccharides.[7]

The serum samples were analyzed for total protein by the biuret reaction described by Weichselbaum[8] and for total glycoprotein hexose by the tryptophan method of Shetlar et al.[9] Paper-strip electrophoresis techniques, as previously described,[10] were utilized for studies of serum protein and glycoprotein fractions. The protein strips were stained with bromphenol blue and quantitated on the Spinco Analytrol Model RB, using 500 mμ interference filters. The glycoprotein strips were stained, using the periodic acid-Schiff reaction, and quantitated on the Analytrol, using 550 mμ interference filters.

EXPERIMENT B.—Twelve rats, Holtzman males, weighing 235 to 265 gm. (approximately 9 weeks of age), were divided into two equal groups. Each rat received subcutaneous injections of 0.5 ml. of turpentine solution (1 part turpentine N.F. to 1 part cottonseed oil). Group 5 received no other treatment. Group 6 received subcutaneous injections of 5 mg. of cortisone acetate twice daily for three days in addition to the turpentine. Seventy-two hours after the initial injection, each animal was killed, as in Experiment A.

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From the Endocrinology Section, Oklahoma Medical Research Foundation, and the Departments of Biochemistry, Medicine, and Pathology, Oklahoma University School of Medicine.

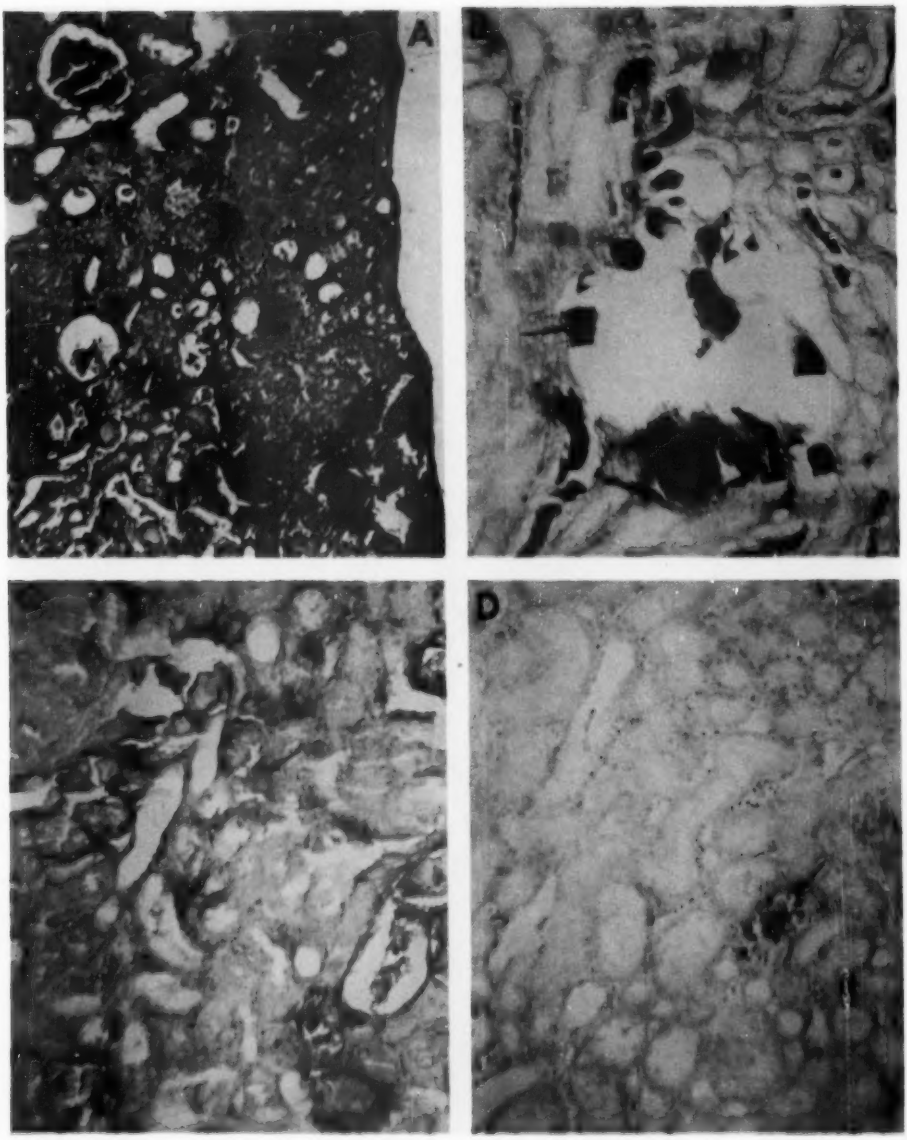

Fig. 1. — These kidney sections (not decalcified) were prepared from rats to which 0.45 ml. of parathyroid extract was administered subcutaneously twice daily for three days. They were killed 72 hours after the initial injection. (A) Dark areas (arrow) showing regions of calcification in tubular epithelium and interstitial tissue. Hematoxylin-eosin stain. (B) Darkly stained areas (arrow) involving large calcified lesions, with disruption of structure. There are also scattered deposits within the tubular-lumens. Periodic acid-Schiff stain for neutral polysaccharides. (C) Numerous blue areas (arrow), indicative of acid polysaccharides, corresponding well with areas of calcification. (D) Kidney section D was prepared from a rat to which 5 mg. of cortisone acetate was administered subcutaneously twice daily for three days, in addition to the parathyroid extract. Only one small Alcian-blue-positive lesion (arrow) after cortisone treatment. Alcian blue stain. (times 118)

TABLE 1.—*Effect of Parathyroid Hormone, Cortisone, and Turpentine on Serum Protein and Glycoprotein* *

Group	No. of Rats	Total Protein, Gm/100 Ml.	Total Glycoprotein, Mg. Hexose/100 Ml.	Glycoprotein/Protein Mg. Hexose/Gm. Protein
I (Control)	6	5.70±0.05 †	184± 7	2.97±0.01
II (Cortisone)	6	5.47±0.04	163± 8	2.99±0.03
III (PTE)	6	5.58±0.01	264± 4 ‡	4.73±0.03 ‡
IV (Cortisone & PTE)	6	5.37±0.19	180±11	3.34±0.05 ‡
V (Turpentine)	6	5.39±0.10	193± 6	3.59±0.04 §
VI (Cortisone & turpentine)	6	6.32±0.04 ‡	245±11 ‡	3.82±0.20 §

* Parathyroid extract, 0.45 ml., was injected subcutaneously twice daily for three days; cortisone acetate, 5 mg., was injected subcutaneously twice daily for three days; turpentine solution, 0.5 ml., was given in a single subcutaneous injection.

† Number following each mean is the standard error.

‡ Significantly different, at 1% level, from the control value.

§ Significantly different, at 1% level, from the control; no significant difference between Groups V and VI.

Results

Table 1 indicates that the serum total protein was not significantly changed by cortisone (Group II), or PTE (Group III), or a combination of these two (Group IV). The serum total glycoprotein was markedly increased by the administration of PTE, as previously reported.[2] However, no significant elevation of serum total glycoprotein occurred in Group IV, which received cortisone and PTE simultaneously. The polysaccharide ratios (glycoprotein/protein), computed for the six groups, are also presented in Table 1. PTE treatment increased this ratio strikingly, as previously reported.[2] Although simultaneous administration of cortisone with the PTE decreased the ratio considerably, a statistically significant elevation, relative to the control, persisted. Turpentine solution also elevated this ratio, as previously reported.[3] No significant difference in this ratio was obtained between the turpentine- and the turpentine-cortisone-treated rats.

The data presented in Table 2 demonstrate the effect of PTE on the distribution of protein among the albumin and globulin fractions of the serum. The albumin fraction is markedly and significantly depressed to less than one-half the normal value. At the same time, there is a significant increase in the α_1-, α_2-, and β-globulin fractions. Cortisone given with PTE largely reversed these effects, since the difference between the results obtained for the corresponding protein fractions of Groups I and IV are not significantly different statistically.

The amounts of glycoprotein (measured as hexose) migrating with the individual protein fractions of the serum are presented in Table 3. Cortisone treatment alone, at this dose level, had little effect on the glycoprotein fractions. On the other

TABLE 2.—*Effect of Parathyroid Extract and Cortisone on the Fractions of the Serum Protein* *

	Concentration (Gm/100 Ml.)				
		Globulin			
Group	Albumin	α_1	α_2	β	γ
I (Control)	3.09±0.02 †	0.97±0.06	0.53±0.06	0.77±0.04	0.35±0.04
II (Cortisone)	3.08±0.15	0.71±0.02	0.64±0.03	0.69±0.02	0.35±0.02
III (PTE)	1.24±0.13 ‡	1.49±0.06 ‡	1.28±0.11 ‡	1.14±0.03 ‡	0.43±0.04
IV (Cortisone & PTE)	2.67±0.14	0.80±0.08	0.79±0.05	0.81±0.05	0.30±0.04

* Parathyroid extract, 0.45., was injected subcutaneously twice daily for three days; cortisone acetate, 5 mg., was injected subcutaneously twice daily for three days. Data shown here were obtained by paper electrophoresis.

† Number following each mean is the standard error.

‡ Signficantly different, at 1% level, from the control.

TABLE 3.—*Effect of Parathyroid Extract and Cortisone on the Fractions of the Serum Glycoprotein* *

	Concentration (Mg. Hexose/100 Ml.)				
		Globulin			
Group	Albumin	α_1	α_2	β	γ
I (Control)	33.1±2.6 †	78.6± 3.9	30.4±1.3	34.5±1.5	7.6±1.7
II (Cortisone)	27.7±4.4	60.0± 4.2	45.1±1.0	25.4±1.6	5.2±0.2
III (PTE)	7.0±1.2 ‡	131.5± 2.6 ‡	55.2±5.7 ‡	64.3±4.0 ‡	5.5±1.2
IV (Cortisone & PTE)	31.2±2.6	68.3±10.0	35.8±2.7	36.2±2.4	8.3±0.5

* Parathyroid extract, 0.45 ml., was injected subcutaneously twice daily for three days; cortisone acetate, 5 mg., was injected subcutaneously twice daily for three days. Data shown were obtained by paper electrophoresis.

† Number following each mean is the standard error.

‡ Significantly different, at 1% level, from the control.

hand, PTE treatment led to a marked decrease in the albumin glycoprotein fraction and an increase in the globulin fractions. Simultaneous administration of cortisone with PTE reversed these PTE effects, since there is no significant difference between corresponding fractions of Groups I and IV.

The effect of cortisone on the renal calcification produced by PTE is presented in Table 4. The dose of PTE administered consistently produced pronounced renal calcification, as illustrated in Figures 1 and 2*A*. When stained sections from the kidneys of the rats in Group IV were compared with those of Group III, it was apparent that cortisone, when given with PTE, was effective in materially reducing the renal calcifying effect of PTE, as demonstrated in Figures 1*D* and 2*B*. This is in agreement with the findings of Laron et al.[1] This effect was further substantiated in the present study by the use of the Kóssa staining reaction.

Renal polysaccharide lesions (stained by the PAS technique), which were present after PTE administration[2] (Fig. 1*B*), were found only occasionally when cortisone was administered with the PTE. Similarly, Alcian-blue-positive material was only slightly increased above normal, and was noted only in those few areas where calcification occurred, as illustrated in Figure 1*D*. Some variation was noted in the amount of calcification in the six cortisone-PTE-treated animals. More renal calcification occurred in one animal than in the other five, but even in this case calcification was much less than that observed in the animals treated only with PTE.

Comment

In agreement with the report of Laron et al.,[1] cortisone was found to antagonize the effects of PTE on calcification in the kidney. In the same animals, cortisone also prevented the elevated serum total glyco-

TABLE 4.—*Effects of Parathyroid Extract and Cortisone on Rat Kidney as Determined Histochemically* *

	Staining Technique (with Component Studied)			
Group	H. & E. (Calcification)	Kóssa (Calcification)	PAS (Polysaccharide)	Alcian Blue (Acid Mucopolysaccharide)
I (Control)	--	--	Normal	Normal
II (Cortisone)	--	--	Slight increase	Normal
III (PTE)	+++++	+++++	Marked increase (Many casts)	Marked increase
IV (Cortisone & PTE)	+ to ++	+ to ++	Slight to moderate increase (some casts)	Slight increase

* Parathyroid extract, 0.45 m. was injected subcutaneously twice daily for three days; cortisone acetate, 5 mg., was injected subcutaneously twice daily for three days.

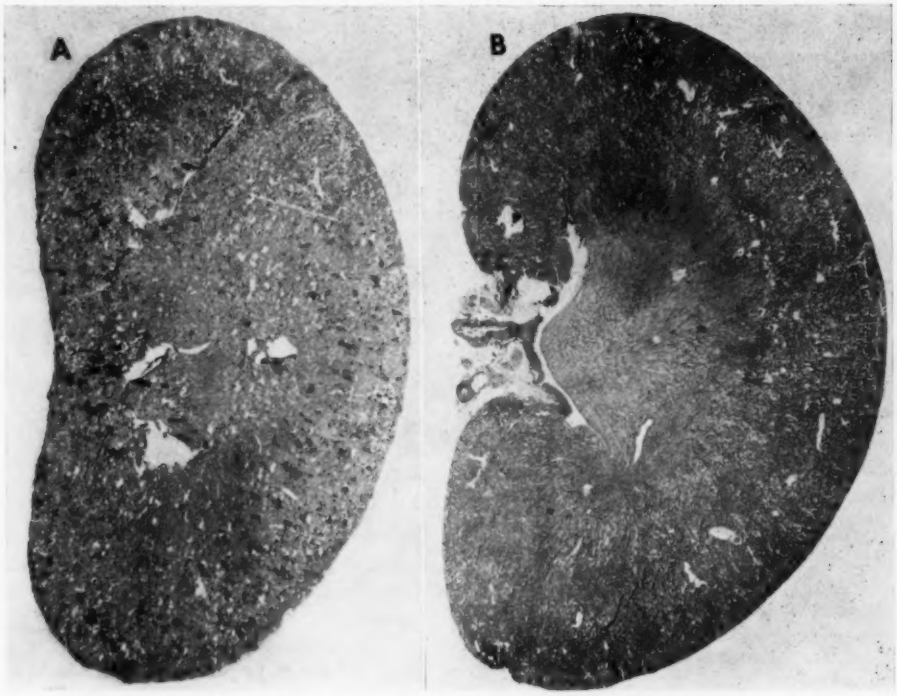

Fig. 2.—These kidney sections (not decalcified) were prepared from rats to which 0.45 ml. of parathyroid extract was administered subcutaneously twice daily for three days. The animals were killed 72 hours after the initial injection. Kidney Section B was prepared from a rat to which 5 mg. of cortisone acetate was administered subcutaneously twice daily for three days, in addition to the parathyroid extract. *A* and *B* were stained with hematoxylin and eosin. (*A*) Note numerous dark areas of calcification throughout kidney, especially in cortex. (*B*) Note that very few dark areas denoting calcification are present in the entire kidney after cortisone treatment. × 7.5.

protein levels and the redistribution among the electrophoretic fractions of serum protein and glycoprotein caused by administration of PTE. This is in contrast to the situation in which cortisone fails to prevent the elevation of serum glycoprotein caused by the subcutaneous injection of turpentine. The effects of PTE may differ, therefore, from the nonspecific inflammatory effects of turpentine in regard to alterations of glycoprotein in the serum.

The mechanism of the antagonistic effects of cortisone on PTE is, at present, only conjectural. It appears possible, however, that this antagonism might be partially explained on the basis of the opposing action of the two hormones on the bone matrix. Follis [11] has described the histological changes in growing rat bone following cortisone administration. He interpreted these changes as being the result of decreased osteolytic activity. One possible explanation of this decreased osteolytic activity is that there may be a change in the metabolism of some mucopolysaccharide component of the matrix. That cortisone does, in fact, alter mucopolysaccharide metabolism has been demonstrated both in vitro and in vivo by Layton. He has shown that cortisone inhibits sulfate esterification in the skeletal and heart muscle of embryonic chick tissue grown in tissue culture.[12] In addition, he has reported that the concentration of bound sulfate in the skin of cortisone-treated rats was only one-fourth that of the controls.[13] These results were

interpreted as indicating that chondroitin sulfate synthesis was impaired by cortisone administration. Layton's observations on the inhibition of sulfur-35 (S^{35}) incorporation by cortisone administration were confirmed and supplemented, both in vitro and in vivo, by Boström and co-workers.[14] After administering sodium sulfate-S^{35}, the chondroitin sulfuric acid isolated from the costal cartilage of cortisone-treated rats was found by these investigators to contain only about 65% of the radioactivity present in that isolated from the control group. Boström and associates have interpreted their results as indicating that cortisone administration has resulted in a decrease in the rate of renewal of the sulfate group of chondroitin sulfate.

On the other hand, it has been postulated by Engel[15] that PTE increases osteolytic activity by causing increased destruction of the bone matrix. Recent work in this laboratory, utilizing S^{35} has indicated that the administration of PTE results in increased amounts of sulfated mucopolysaccharides in the blood.[16] This observation tends to support the hypothesis that bone matrix is directly affected by PTE. In view of Follis' results, this hypothesis is further strengthened by the data presented here demonstrating that the typical serum glycoprotein elevation and renal calcification found after PTE administration are prevented by simultaneous cortisone treatment.

The effect of PTE on the fractions of serum protein and glycoprotein is pronounced. The serum total protein is not affected by PTE, but the distribution between albumin and globulin fractions is clearly altered. PTE treatment altered both the serum total glycoprotein and the distribution among fractions, as determined by hexose content. Cortisone antagonized this PTE effect also, though cortisone had only a minor effect on these components when given singly. No feasible explanation can be presented at this time to account for this particular action of cortisone. The possibility that cortisone is acting through a mechanism distinct from that involving bone matrix and mucopolysaccharide metabolism cannot be excluded.

Summary

Cortisone administered to rats together with parathyroid extract prevented the elevation of serum total glycoprotein (expressed as bound hexose), which occurred when parathyroid extract was administered alone. Alterations in serum protein and glycoprotein components (decreases in albumin; increases in α_1-, α_2-, and β-globulin) which followed administration of parathyroid extract were also inhibited. Calcification in the kidney (as indicated by hematoxylin-eosin and Kóssa techniques), was prevented by the combined treatment. Cortisone was shown to have essentially no effect on the nonspecific elevation of serum glycoprotein resulting from injection of turpentine.

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Histopathology of Amino Acid Deficiencies

VI. Effect of Arginine Deficiency on the Liver, with Consideration of the Testes and Accessory Sex Glands

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Numerous reports have established that arginine can be synthesized in vivo by the rat.[1-6] It has also been shown that this amino acid is dispensable for the adult rat, since its synthesis proceeds at a rate sufficient to meet normal maintenance requirements, whereas in young rats this maintenance requirement is not met and arginine deficiency results in less than optimum growth.[3] Histological studies of arginine-deficient rats have been limited to the testes, and these reports are in disagreement. Shettles[7] reported histological evidence of serious testicular damage in one rat fed an arginine-deficient ration. Holt and Albanese[8] have reported that arginine deficiency caused degenerative testicular changes in growing rats as early as the third week, which, in the course of two months, became so extensive as to make the tissue difficult to recognize histologically. On the other hand, Williams and Watson[9] mated pairs of adult rats which had been fed an arginine-free diet for 49 days and noted that all females conceived at the first estrus and completed normal gestation. They found no histological evidence of impaired spermatogenesis. Cannon[10] deprived young male rats of arginine for 38 days and noted no testicular damage. Womack and Rose[11] and Sallach, Koeppe, and Rose[12] showed that proline, arginine, and glutamic acid are mutually interconvertible in the rat. Black and Rose[13] and Gunther and Rose[14] reported arginine to be responsible for better weight gains in rats in which proline, arginine, or glutamic acid were added singly as supplement to proline-arginine-glutamic acid-free diets. The present series of experiments includes groups of rats deprived of proline or arginine or of arginine and proline together.

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Materials and Method

Male Sprague-Dawley rats, which reach puberty at 40-50 days, were grouped according to age, and each group was further subdivided into deficient, pair-fed, and normal control subgroups. The normal control rats were fed a purified diet consisting of a mixture of 19 crystalline amino acids, vitamins, sucrose, cottonseed oil, and the necessary minerals as described by Rose, Oesterling, and Womack.[15] The deficient rats received the same diet, complete in every respect, except for the total omission of the amino acid deficiency being investigated. The caloric value of the missing amino acid(s) was supplied by additional sucrose. The pair-fed rats received the complete diet in such amounts that the daily food consumption of each was no greater than that of its deficient partner. Each animal was kept in an individual cage, had free access to water, and, with the exception of the pair-fed rats, was fed ad libitum. All rats were weighed daily.

Since arginine is considered to be an essential dietary component only for immature rats, one experiment was designed to observe what effects, if any, occurred in adult rats for which dietary arginine is considered to be nonessential. A second group was included to observe the effects of an arginine-deficient diet fed to young rats for a short period prior to puberty and for an extended period beyond. A third experiment was planned to study not only the effects of arginine deficiency on young rats prior to puberty, but also the effects of proline and arginine-proline deficiencies, since proline has been shown to be convertible to arginine.[11-14] The rats were grouped as follows:

Group I: 100-day-old rats fed an arginine-deficient diet for 70 days

Group II: 30-day-old rats fed an arginine-free diet for 70 days

Group III: 20-day-old rats fed a proline-, an arginine- or an arginine-proline-free diet for 21 days

At the termination of the respective experimental periods, the animals were killed by exsanguination. At necropsy, tissue samples were removed (liver, adrenals, spleen, stomach, duodenum, pancreas, aorta, testes, epididymides, ventral prostate, seminal vesicles, urinary bladder, ureter, heart, thymus, lymph node, parathyroids and thyroids, trachea, skeletal muscle, pituitary, kidney and tibia) and prepared for histological examination. Only the adrenals, testes, and pituitary of each rat were weighed. All tissues, except the pituitaries, were fixed in Bouin's fixative and stained with hematoxylin and eosin after paraffin sectioning. Pieces of liver were fixed in Gendre's fixative, sectioned, and stained with Best's carmine for glycogen demonstration. Other portions of liver and one adrenal were fixed in neutral formalin, frozen-sectioned, and stained for lipid with oil red O. The pituitaries were fixed in Zenker-formol solution and stained by the aldehyde-fuchsin (AF) and periodic acid-Schiff (PAS) methods. These techniques were developed by Halmi[16] and Purves and Griesbach[17,18] to differentiate pituitary thyrotrophic and gonadotropic basophils. We have described the use of these methods in our earlier studies.[19-21] Sections of pituitary were also stained with acid fuchsin for examination of the acidophils.

Results

The deficient rats in all experimental groups did not have the unkempt appearance and did not suffer seriously from the anorexia usually seen in rats fed ad libitum a diet deficient in an essential amino acid. In Group I, the normal control rats consumed an average of 18 gm. of diet per day. The arginine-deficient animals of this group consumed a daily average of 12 gm. during the first 21 days, 17 gm. during the next 25 days, and 16 gm. during the final 24 days. The normal control rats of Group II ate an average of 13 gm. per day, while the arginine-deficient rats consumed 7 gm. daily during the first 21 days, 9 gm. per day during the second 25 days, and 12 gm. daily during the last 24 days. In Group III, normal control rats ate a daily average of 7 gm. per day, while the average daily food intake of the proline-deficient rats was 6 gm. The arginine- and arginine-proline-deficient animals ate 4 gm. per day.

The Table gives a comparison of the initial and final body weights and the mean weight and percentage of body weight of the adrenals, testes, and pituitary. While the normal control rats of Group I almost doubled their initial weight, the mean weight of the deficient and pair-fed rats increased approximately 70% during the same period. The normal control rats of Group II increased their mean body weight by four times, and the arginine-deficient and pair-fed rats of this group increased 2.5- and 3-fold, respectively. In Group III, the mean body weight increment of the normal control rats was 1.3 times; that of the proline-deficient and their pair-fed mates was insignificantly less, 1.2 times. In the arginine- and arginine-proline-deficient subgroups, the mean body weight gain was 63% and 77% respectively. That of their pair-fed controls was 85% and 92% respectively. No significant differences exist between the mean percentage of body weight of the adrenals, testes, or pituitaries of the various groups of arginine-deficient and pair-fed rats.

Of all the tissues examined histologically, only the liver showed notable evidence of alteration. The livers of all arginine-deficient rats of Groups I and II had extensive periportal lipidosis (Figs. 1, 2). In Group III, nine of the arginine-deficient and four of the arginine-proline-deficient rats showed the presence of periportal liver fat (Fig. 3). The livers of the proline-deficient rats of this group were normal in all respects. Liver lipidosis was not present in any group of pair-fed or normal control rats.

The testes, epididymides, seminal vesicles, and ventral prostates of the deficient, pair-fed and normal control rats of all groups were normal and showed no signs of experimental deterioration. The testes of all deficient, pair-fed and normal control rats in Groups I and II had normal seminiferous tubules with active spermatogenesis and spermiogenesis (Figs. 4-9). These testes

Summary of Mean Body Weights and Organ Weights of Arginine-, Proline-, and Arginine-Proline-Deficient Rats

Group	No. of Rats	Initial Age, Days	No. Exper. Days	Mean Body Weight, Gm.		Mean Organ Weight, Mg., and Mean % of Body Weight					
						Adrenals (2)		Testes (2)		Pituitary	
				Initial	Final	Mean Wt.	% B.W. *	Mean Wt.	% B.W. *	Mean Wt.	% B.W. *
Group I											
Normal control	2	100	70	188	353	56±0.6 †	0.15±0.002 †	1,366±72 †	4.0±0.25 †	12.5±0 †	0.04±0.0005 †
Arginine-deficient	10	100	70	192	329	52±3.0	0.15±0.005	1,644±59	5.0±0.08	12.9±0.3	0.04±0.005
Pair-fed control	10	100	70	192	326	52±2.0	0.15±0.002	1,742±44	5.3±0.09	12.5±0.5	0.04±0.007
Group II											
Normal control	2	30	70	50	258	44±4	0.16±0.01	1,623±28	6.3±0.05	12.6±0.05	0.05±0.0005
Arginine-deficient	10	30	70	55	192	36±1	0.18±0.06	1,312±36	6.8±0.18	7.9±0.3	0.04±0.002
Pair-fed control	10	30	70	54	217	38±1	0.17±0.004	1,507±45	6.9±0.23	9.2±0.5	0.04±0.002
Group III											
Normal control	5	20	21	42	99	25±0.5	0.25±0.01	1,394±101	14±0.5	5.0±0.5	0.05±0.005
Proline-deficient	10	20	21	41	92	23±0.7	0.25±0.02	1,302±56	14±0.7	4.7±0.2	0.05±0.002
Proline pair-fed	10	20	21	41	92	25±0.7	0.27±0.06	1,369±45	15±0.4	5.8±0.3	0.06±0.002
Arginine-deficient	10	20	21	41	67	21±0.9	0.32±0.03	935±69	14±0.7	4.1±0.3	0.06±0.003
Arginine pair-fed	10	20	21	41	76	24±0.7	0.32±0.02	1,135±24	15±0.5	4.7±0.1	0.06±0.001
Arginine-proline-deficient	10	20	21	40	70	23±0.9	0.32±0.03	1,067±64	15±0.6	3.7±0.2	0.05±0.004
Arginine-proline-pair-fed	10	20	21	40	77	25±0.7	0.32±0.01	1,105±43	15±0.4	5.0±0.2	0.06±0.003

* $\times 10^{-1}$.

† Standard error of mean.

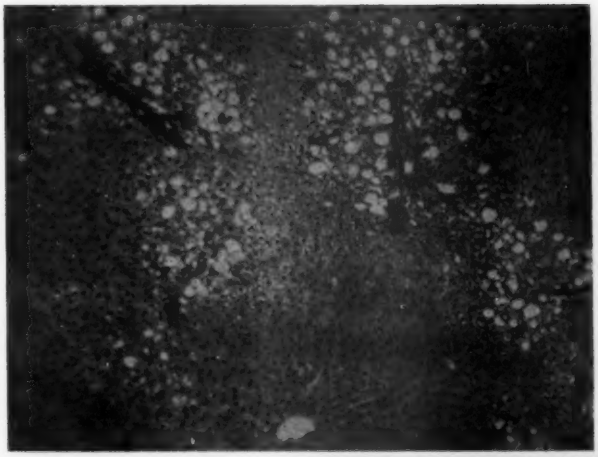

Fig. 1.—Liver of an adult rat fed an arginine-deficient diet for 70 days (Group I). Note the extensive periportal lipidosis. Hematoxylin and eosin; × 70.

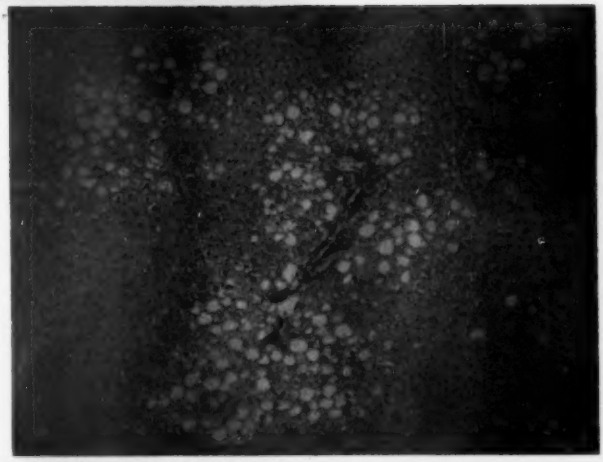

Fig. 2.—Liver of a rat fed an arginine-deficient diet for 70 days, commencing at 30 days of age (Group II). Note the periportal lipidosis. Hematoxylin and eosin; × 70.

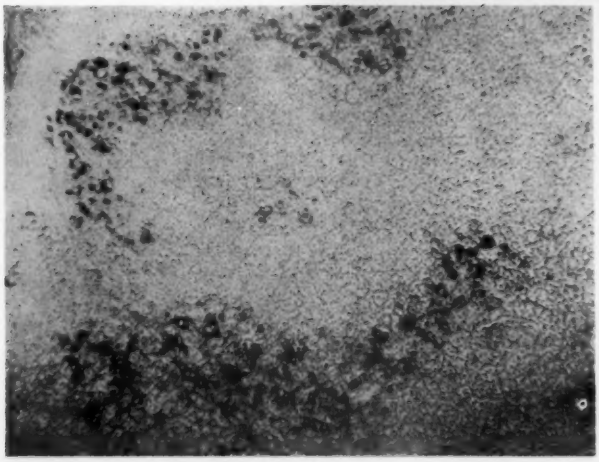

Fig. 3.—Liver of a young rat fed an arginine-deficient diet for 21 days (Group III). Note the perilobular distribution of fat. Frozen section; oil red O; × 70.

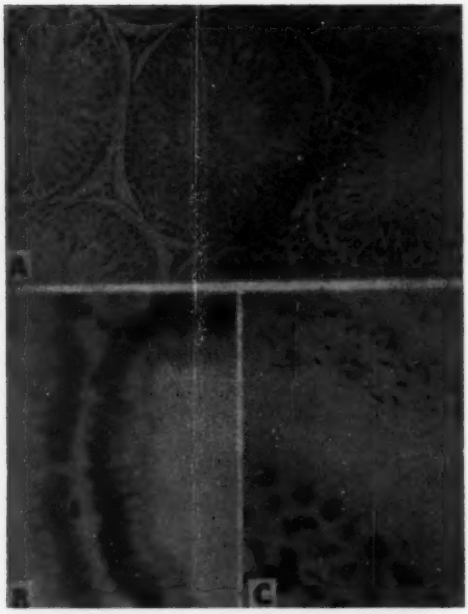

Fig. 4.—Testis (*A*), ventral prostate (*B*), and epididymis (*C*) of an adult normal control rat of Group I. Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

produced normal sperm cells, which were present in large number in the epididymides (Figs. 4-9). The testicular interstitial cells were morphologically normal and physiologically active, as evidenced by the normal secretory epithelium of the ventral prostates (Figs. 4-9) and seminal vesicles.

The testes of the proline- arginine- and arginine-proline-deficient rats of Group III, as well as those of the pair-fed and normal

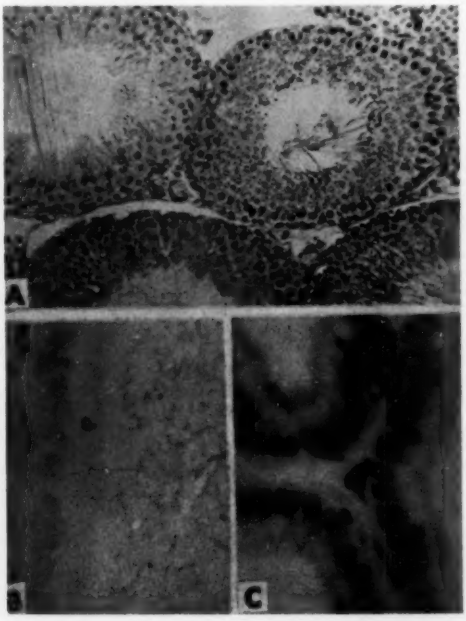

Fig. 5.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of an adult rat fed an arginine-deficient diet for 70 days (Group I). Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

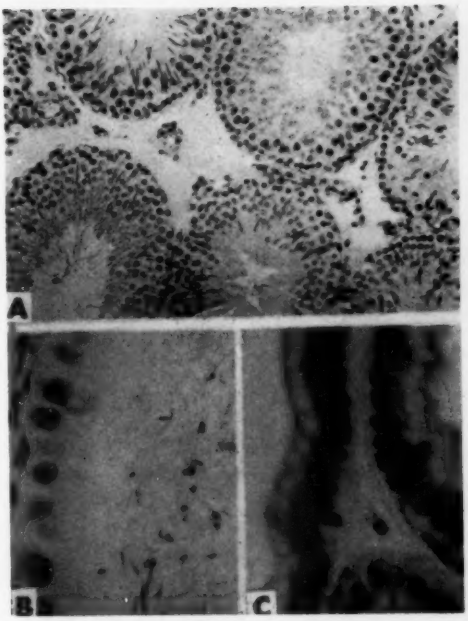

Fig. 6.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of an adult pair-fed rat of Group I. Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

controls, were normal for rats of that age (41 days), approaching puberty. The seminiferous tubules of the deficient and pair-fed rats were slightly narrower than those of the normal controls rats, but otherwise showed no significant differences. Spermatogenesis was becoming active, but spermiogenesis was incomplete. No mature or free sperm cells were visible in either the testes or the epididymides, but numerous sloughed, degenerat-

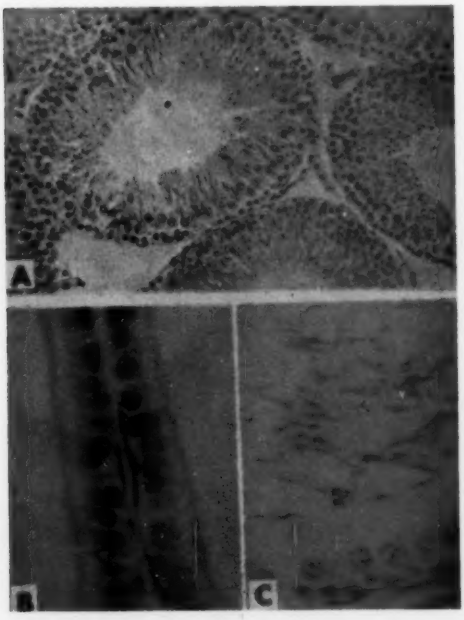

Fig. 7.—Testis (*A*), ventral prostate (*B*), and epididymis (*C*) of a normal control rat of Group II. Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

Fig. 8.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of an arginine-deficient rat of Group II. Hematoxylin and eosin; (*A*) ×70; (*B*), (*C*) ×344.

ing testicular cells were visible in both organs (Figs. 10-16). The presence of such cellular debris is normal in rats nearing sexual maturity. The prostatic and seminal vesicular epithelium of all deficient, pair-fed, and normal control rats was histologically normal (Figs. 10-16), although the prostatic acini of the arginine- and arginine-proline-deficient rats were smaller than normal acini.

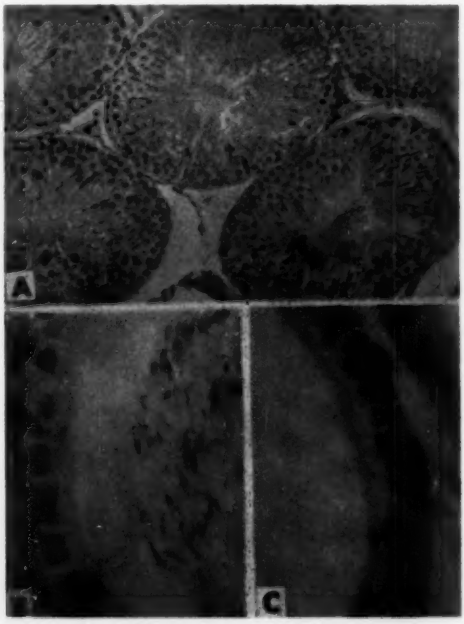

Fig. 9.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of a pair-fed rat of Group II. Hematoxylin and eosin; (*A*) ×70; (*B*), (*C*) ×344.

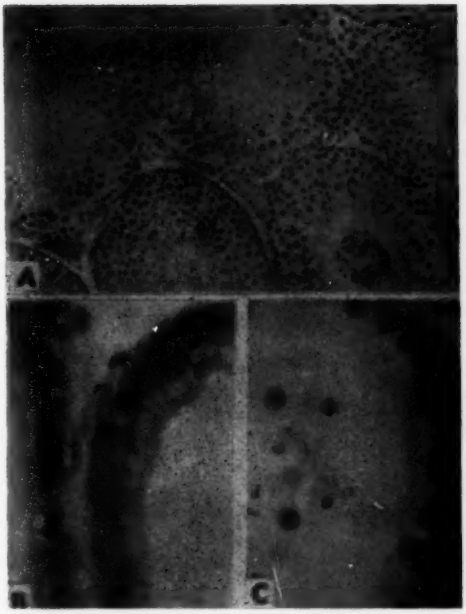

Fig. 10.—Testis (*A*), ventral prostate (*B*), and epididymis (*C*) of a normal control rat of Group III. Hematoxylin and eosin; (*A*) ×70; (*B*), (*C*) ×344.

Comment

The results of this series of experiments demonstrate that the total absence of dietary arginine, or arginine and proline, has a distinct effect upon the liver, but no effect on the testes or accessory sex glands of immature and mature rats. The periportal accumulation of liver fat is similar to that observed by others in amino acid deficiency studies.[23-25] It is of interest to note that

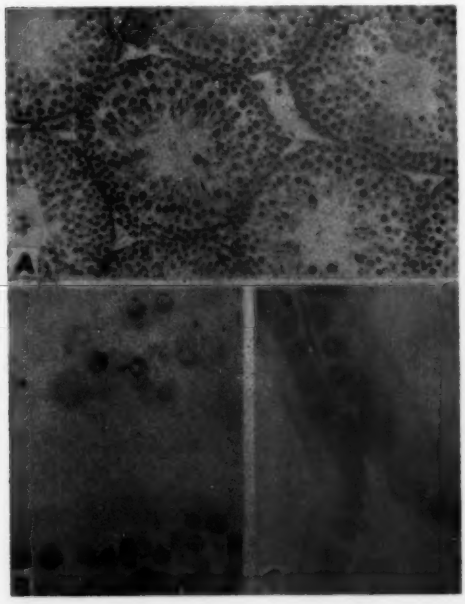

Fig. 11.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of a young rat fed a proline-deficient diet for 21 days (Group III). Hematoxylin and eosin; (*A*) ×70; (*B*), (*C*) ×344.

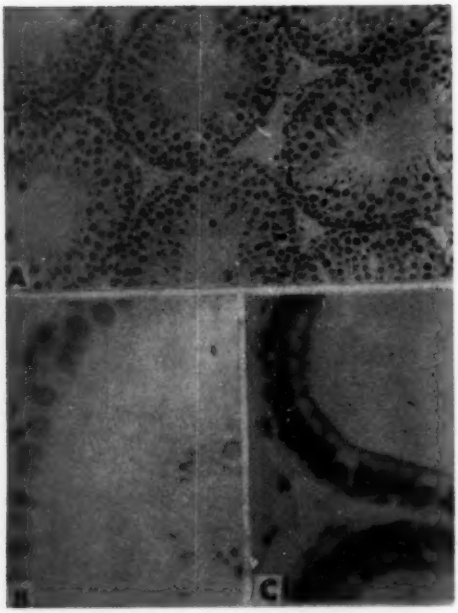

Fig. 12.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of a pair-fed rat of the proline-deficient subgroup (Group III). Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

these reports describe periportal liver lipidosis in animals deficient in an essential amino acid, while we observed that, although arginine has not been considered essential for mature rats, feeding an arginine-deficient diet to such rats resulted in fatty livers. The presence of lipidosis in 65% of the combined arginine- and arginine-proline-deficient rats (Group III) showed that such change could occur even in young

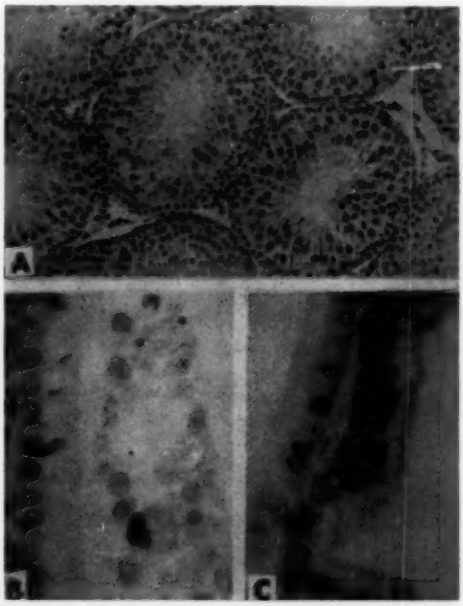

Fig. 13.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of a young rat fed an arginine-deficient diet for 21 days (Group III). Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

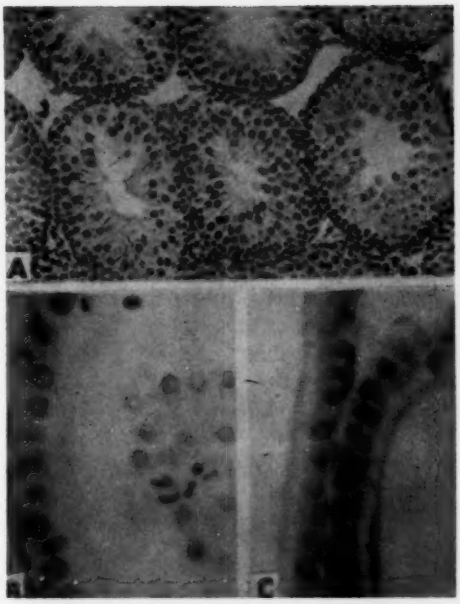

Fig. 14.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of a pair-fed rat of the arginine-deficient subgroup (Group III). Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

rats deprived of arginine, despite the fact that their body weight increased, although at a rate less than optimum, and notwithstanding the fact that their daily food consumption was only 57% that of the normal control. It was also noted that, since liver lipidosis was present in all deficient rats of Group II, this condition persisted and progressed with continued arginine deprivation. The accumulation of periportal liver fat in

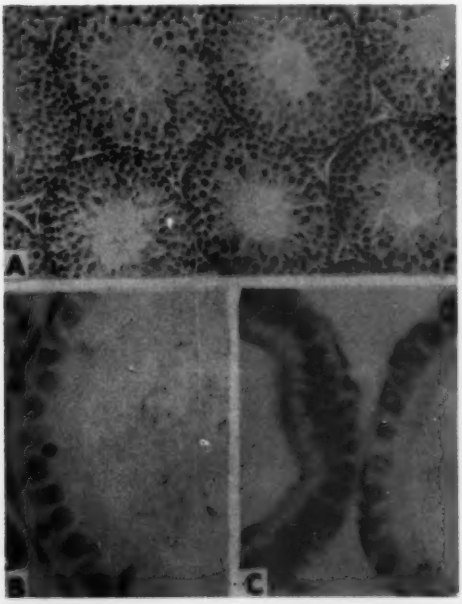

Fig. 15.—Testis (*A*), epididymis (*B*), and ventral prostate (*C*) of a young rat fed an arginine-proline-deficient diet for 21 days (Group III). Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

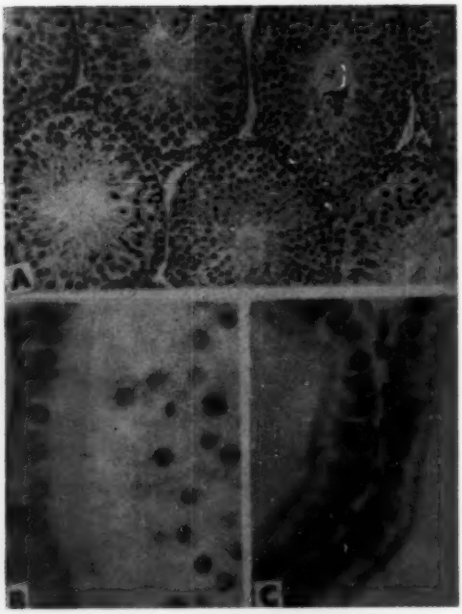

Fig. 16.—Testis (A), epididymis (*B*), and ventral prostate (*C*) of a pair-fed rat of the arginine-proline-deficient subgroup (Group III). Hematoxylin and eosin; (*A*) × 70; (*B*), (*C*) × 344.

the deficient rats of Group I suggests that, from histological evidence, this amino acid may be indispensable for normal liver function in adult rats.

Shettles' report[7] of testicular degeneration in the rat fed an arginine-deficient diet cannot be of significance, since his report is based upon one experimental animal. The results of the present study are not in agreement with those of Holt and Albanese,[8] who reported serious testicular damage in arginine-deficient rats. Our observations substantiate those of Williams and Watson[9] and Cannon[10] that arginine deficiency is without effect upon the testes of the immature or adult rat.

Although there appear to be differences between the mean weights of the adrenals, testes, and pituitaries of deficient and pair-fed rats, when compared on the basis of percentage of body weight the differences are not significant.

Summary

The total omission of dietary arginine or both arginine and proline resulted in periportal liver lipidosis in young and adult male rats. The livers of proline-deficient rats were unaffected. None of these dietary deficiencies produced changes in the testes or accessory sex glands.

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Beta Aminopropionitrile Concentration and Angiorrhexis in Rats

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Feeding Lathyrus odoratus meal or β-aminopropionitrile (BAPN) in proper concentration to weanling rats regularly produces some degree of skeletal deformity,[1-5] whereas the incidence of angiorrhexis,[4-7] hindlimb paralysis,[1,2,7,8] herniation,[2,5,6] and weight gain may be variable. Differences in tissue response have been attributed to protein concentration,[5,6,10,11] supplements of amino acids,[13] and the concentration of the lathyrogenic agent in the diet.[1,2] Correlation of specific tissue changes, such as herniation, hindlimb paralysis, or angiorrhexis, with the concentration of the lathyrogenic agent in the diet is not fully resolved, because other factors such as age,[1,4] sex,[14] species,[15,16] and period of feeding also influence the tissue response of growing animals.

It is to be anticipated that when tissue response can be modified by so many variables controversy will exist as to whether any modification in response is due to the protein, the concentration of lathyrogenic factor, or some other unknown factor. This is particularly true in experiments in which sweet-pea-meal diets were fed and the concentration of the lathyrogenic agent was not known.[17] It has been established that BAPN will produce all of the tissue changes which are encountered after the feeding of Lathyrus odoratus meal.[8,9,13,18] Because of this, it is now possible to feed a variety of diets with the addition of varying amounts of BAPN and evaluate the tissue response in terms of the concentration of the lathyrogenic agent in the diet. In this study a commercial diet was fed to rats in order to evaluate the effect of varying concentrations of BAPN upon the development of aortic rupture and skeletal deformity. In several assays compounds were fed with mono-β-aminopropionitrile fumarate, and their influence on inhibiting or accelerating the development of lathyritic lesions was noted.

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Department of Pathology, University of Wisconsin Medical School.

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Method

Female Sprague-Dawley rats, weighing 39-46 gm., were used for these assays. All animals were fed Purina Rat Pellets.* Chemicals fed to the rats were dissolved in a minimum of water, buffered to pH 7 when necessary, and diluted to 100 ml. in 10% ethanol. Either a 1% or 2% concentration of each chemical was stored at 4 C before mixing. Final dilutions of each compound were made daily by the addition of proper volumes of the concentrated solutions to a requisite volume of drinking water. Mono-β-aminopropionitrile fumarate (BAPN)† was used in these assays. Food and water were fed ad libitum to the rats. Five to six rats were housed in one open-bottomed mesh cage for periods of 37 to 84 days. When the rat died, or after a specified interval, autopsy was performed. The following data were recorded for each rat: weight gain; presence of sternal, vertebral column, and femoral deformities; fibrosis, aneurysms, or perforation of the aorta, and any other abnormal alteration. Since aortic perforations invariably occur in the aortic arch in this strain of rats, only the heart and thoracic aortas were fixed in 10% formalin prior to microscopic examination.

Gross Observations

In estimating skeletal deformity, the following method was employed: The severity of malformations in the sternum, vertebral

* Ralston Purina Mills.

† Generously supplied by Abbott Laboratories, North Chicago, Ill.

Fig. 1.—Examples of femurs used in estimating skeletal deformity are shown arranged in order of increasing severity of deformity from left to right; normal (*0*), minimal (*1*), moderate (*2*), severe (*3*). A similar method of estimating deformities in the sternum and vertebral column was also employed in calculations of numerical skeletal deformity in the different assays.

column, and femur was evaluated and expressed numerically for each animal. Numerical expressions of deformity were as follows: normal, 0; minimal, 1; moderate, 2; severe, 3 (Fig. 1). The sum of the numbers of estimated deformities for one group divided by the sum of structures (total of sternums, vertebral columns, and femurs) examined is recorded as a numerical average for the deformities in that assay. For example, if minimal deformities were found in 15 structures from 5 rats, then the total numerical deformity of 15 would be divided by a total of 15 structures to equal 1. A numerical value of 1 or less is considered to be an expression of minimal skeletal deformity for a given assay.

A total of 10 assays on 16 control and 40 test rats were performed in this study. Significant data, such as the dose of BAPN, supplement when fed, the period of feeding, the weight gain, an approximation of skeletal deformities, aortic rupture, and aortic fibrosis are included in the accompanying Table.

Fig. 2.—Heart and thoracic aorta of a test rat which died suddenly of aortic rupture. The dissecting hemorrhage and associated hematoma were always present in the region of the aortic arch.

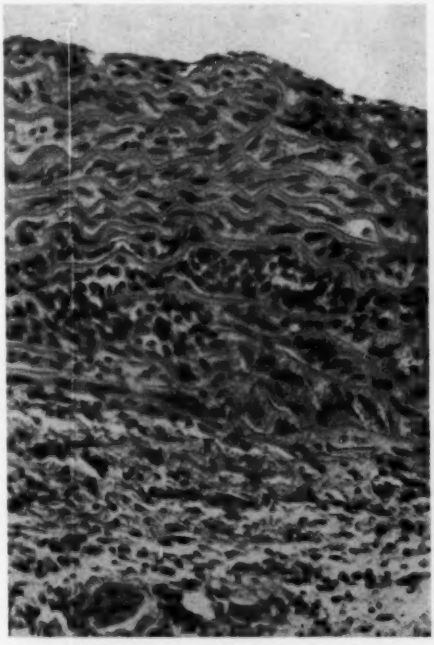

Fig. 3.—Formalin-fixed thoracic aorta from a test rat. There is evidence of minimal medial fibrous proliferation. In cases of rapid medial hemorrhagic dissection, such as occurred in this study, fibrosis of the aorta is usually absent or minimal.

Rats fed for 84 days (Assays 1 and 2) show less weight gain than other rats which were fed for shorter periods of time. This is due to diminished weight gain, which occurs after 50 days of feeding. Weight gains in these two groups were similar. At 120 mg. of BAPN per 100 ml. of drinking water the toxic effects are minimal. None of the rats in Assay 2 died of angiorrhexis. Microscopic examination of the thoracic aorta, however, revealed moderate to severe fibro-

Effect of BAPN-Fumarate Concentration on the Development of Aortic Rupture and Skeletal Deformity in Weanling Rats

Assay No.	Drugs Fed in Drinking Water	Concentration in Drinking Water, Mg/100 Ml.	Days Fed	Weight Gain, Gm/Day	Died Aortic Rupture	Numerical Average Skeletal Deformity	Microscopic Fibrosis Aorta
1	Control	--	84	2.0	0/5 *	0.0	0/5
2	BAPN	120	84	2.0	0/6	0.4	3/6
3	Control	--	42	3.4	0/6	0.0	0/6
4	BAPN	150	42	2.6	2/6	0.9	4/6
5	BAPN	150	42	2.4	3/6	1.1	5/6
	DL-methionine	300					
6	BAPN	150	42	2.2	3/6	1.0	5/6
	Salicylaldehyde	80					
7	BAPN	150	42	3.2	0/6	0.6	3/6
	Pyridoxine HCl	50					
8	Control	--	37	3.6	0/5	0.0	0/5
9	BAPN	200	37	2.2	3/6	0.9	4/6
10	BAPN	200					
	Pyridoxine HCl	100	37	2.4	4/6	1.2	2/6

* Number of rats which died of aortic rupture.

sis in three of six test rats. Skeletal deformity expressed numerically was equal to 0.4. Most of the vertebral columns and sternums in the test rats were normal. Deformities were limited principally to enlarged diaphyses and fibrous hyperplasia of the proximal femurs in Assay 2.

In Assays 4, 5, 6, and 7, when 150 mg. of BAPN per 100 ml. was fed, the test rats began to die of angiorrhexis. Weight gains were less in rats fed BAPN when compared with the controls in Assay 3. Supplements of DL-methionine and salicylaldehyde which were fed with BAPN in drinking water, were not protective. None of the rats in Assay 7 died, suggesting that pyroxidine (vitamin B6) might have some protection upon aortic involvement. Even though none of the rats in Assay 7 died of angiorrhexis, microscopic examination revealed significant fibrosis in the thoracic aorta in three of six rats. Numerical expressions of skeletal deformity varied from 0.6 to 1.1 in Assays 4-7. In Assay 4, minimal to moderate sternal deformities were observed in four of six rats. Two of six vertebral columns had minimal deformities, whereas all of the femurs were deformed in this group. Observations of the sternums and vertebral columns of rats in Assays 5, 6, and 7 did not differ appreciably from those observed in Assay 4.

In Assays 9 and 10, when 200 mg. of BAPN per 100 ml. was included in the drinking water, many of the rats died of angiorrhexis. Numerical expressions of skeletal deformity were comparable to those encountered in Assays 4, 5, and 6. In Assay 10, when pyridoxine was increased to 100 mg. per 100 ml. of drinking water, there was no evidence of protection against angiorrhexis or skeletal deformities. Similarities in response in Assays 4 and 9 are attributable to the fact that in Assay 9 rats ingested roughly 25% less water. None of the rats in any of the test assays developed hernias or hindlimb paralysis.

Comment

Studies of experimental lathyrism indicate that multiple variables function in the survival of animals and the production of tissue changes. Commonly accepted factors which modify tissue response are age, sex, species, concentration of the lathyrogenic agent, and the duration of feeding. Other probable variables which have not been fully evaluated are concentration and source of protein, as well as individual animal variability in response to BAPN feeding. Owing to the presence of so many variables, it is extremely difficult to determine which one of these may be responsible for specific tissue

changes. Studies in which comparisons of weight gain in control and test animals have not been made are of dubious value because it is not possible to resolve whether poor weight gains are due to the toxicity of BAPN or to a deficiency of some essential ingredient in the diet. Comparisons of weight gains in control and test rats in previous studies by ourselves and others suggest that the feeding of the lathyrogenic agent was frequently complicated by feeding proteins either of poor biological value or of good value at inadequate concentrations.[4-6] In this study there is ample evidence from the weight gains in the control rats to show that the diet is adequate and that tissue response is due to differences in BAPN concentration.

The concentration of BAPN in the diet, as one might anticipate, is of critical importance to the development of tissue changes and weight gains. In this study a slight increase (120 to 150 mg. of BAPN per 100 ml. of drinking water) of the lathyrogenic agent was followed by the production of angiorrhexis. When BAPN was increased further to 200 mg. per 100 ml., the rats restricted their fluid intake and the tissue response was comparable to the 150 mg. level. Strange to say, most of the test rats that died showed weight gains in excess of 2.1 gm. per day and failed to develop severe skeletal deformities, herniation, or hindlimb paralysis.

In previous studies done in this laboratory the lathyrogenic factor was fed with low (12%) casein diets because the incidence of aortic rupture was markedly depressed by increasing the casein concentration to 18%.[5,6] Why low-casein levels in the diet predispose to the development of aortic rupture is not known. Whether differences in tissue response observed in different laboratories are due to low concentrations or poor biologic value of the protein which was fed or to the concentration of BAPN employed is not apparent. One conspicuous fact is evident that when Purina pellets were fed, the rats died of angiorrhexis before severe skeletal deformities developed. These observations are in agreement with the suggestion of Lee and Dupuy, who proposed that the amino acid composition of proteins may be critical in the development of tissue changes in experimental lathyrism.[11] Purina pellets are calculated to contain not less than 23% protein. It is not apparent why 18% casein is protective against angiorrhexis, whereas Purina pellets at 23% protein are not. Variations in severity of hemorrhage in turkeys due to changes in proteins at a similar concentration of BAPN have also been observed by Waibel and Pomeroy.[12] Observations suggest that variations in tissue response which may be conditioned by the diet fed in association with BAPN have a direct bearing on the mechanism of lathyrus toxicity. This study, along with others, points out before tissue changes are attributed solely to BAPN concentration, individual animal variability, protein concentration, and biologic value must be more fully evaluated.

Summary

Rats fed Purina pellets with BAPN tend to die of angiorrhexis before severe skeletal deformities have a chance to develop. The concentration of BAPN which is fed is critical for the development of angiorrhexis. At concentrations of 200 mg. of BAPN per 100 ml. of drinking water the rats restrict their fluid intake, so that the tissue changes are comparable to the 150 mg. level. It is not apparent from this study why herniation and hindlimb paralysis failed to develop. These observations suggest that before all of the tissue changes are attributed solely to the concentration of BAPN in the diet, the concentration and source of proteins, as well as individual animal variability in response to BAPN feeding, should be analyzed more thoroughly.

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Acute Radiation Effects in the Esophagus

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Introduction

Dysphagia and substernal burning are fairly common complaints in patients who have been subjected to significant thoracic radiation therapy. In spite of these symptoms of radiation esophagitis, the effects of radiation in the esophagus have been of little interest to either radiologists or pathologists. It has commonly been felt that the esophagus is relatively radioresistant. Desjardins,[1] in 1931, noted that specific radiosensitivity is not great in the esophagus, and Warren and Friedman,[2] in their review article on effects of radiation, in 1942, stated that the response in the esophagus was that of any stratified squamous mucous membrane. Engelstad[3] first called attention to the degenerative changes occurring in the esophagus secondary to irradiation when he studied histologically the changes in both the esophagus and the trachea of dogs following irradiation. A recent article by Seaman and Ackerman[4] illustrates very well, both clinically and pathologically, some of the effects of radiation in the esophagus following betatron therapy. The increasing use of supervoltage radiation therapy suggests that further study of radiation effects in the esophagus is warranted.

Procedure

A series of Sprague-Dawley albino rats was subjected to 3,000 r of radiation to the thorax, with shielding of the remainder of the body. Radiation factors were 250 k.v.; 30 ma.; TSD, 70 cm.; HVL, 1.6 mm. Cu; dose rate 70 r per minute. If one can extrapolate from the Strandqvist[5] curves relating radiation effect to duration of exposure for squamous epithelium, 3,000 r delivered in a single dose is approximately equivalent to 5,000 r fractionated over a four-week period. Animals were killed periodically through the course of the experiment and the esophageal lesions studied histologically. The induction of radiation esophagitis caused significant mortality in the animals and necessitated feeding a liquid diet during the second and third weeks of the experiment; before and after that time the animals were maintained on stock Laboratory Chow. With the institution of liquid feedings during the period of acute radiation esophagitis, mortality in the experiment was reduced to approximately 30%.

During the acute phase of the experiment, animals were killed usually at two-day intervals, whereas later in the course of the experiment the intervals of killing were lengthened. At least two animals were killed for each observation point, and during the periods of rapid destruction and regeneration within the esophagus, six animals were killed at each time interval.

Results

The earliest apparent effect within the esophagus was seen at four days, when there was a slight submucosal congestion and infiltration of leukocytes, which were apparent when the tissues were compared with those of the control animals (Figs. 1 and 2). By six days mucosal necrosis was apparent, with loss of the superficial epithelium, and by the seventh day there was extensive sloughing of the necrotic esophageal mucosa (Fig. 3). At this time there was only slight submucosal inflammation and congestion. The sloughing of necrotic esophageal mucosa resulted in plugging of the esophagus with debris, causing dilatation of the esophagus. The plugging of the esophagus appeared to reach a maximum at about 10 days, at which time there was moderate inflammation both adjacent to the necrotic material and in the muscularis of the esophagus (Fig. 4). At this

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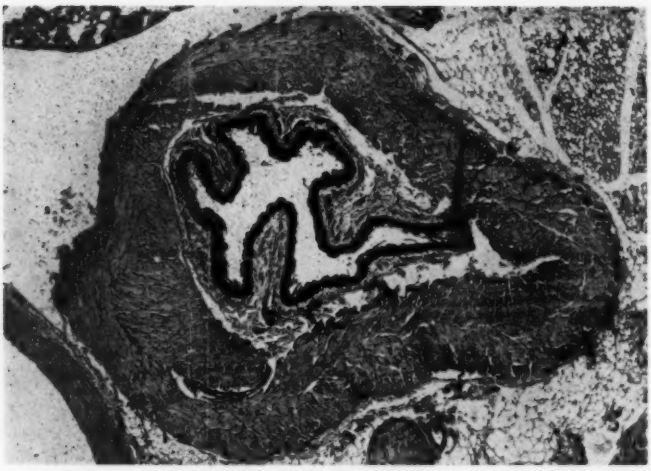

Fig. 1.—Normal esophagus, cut in cross section, from control rat. Reduced to 63% of mag. × 65.

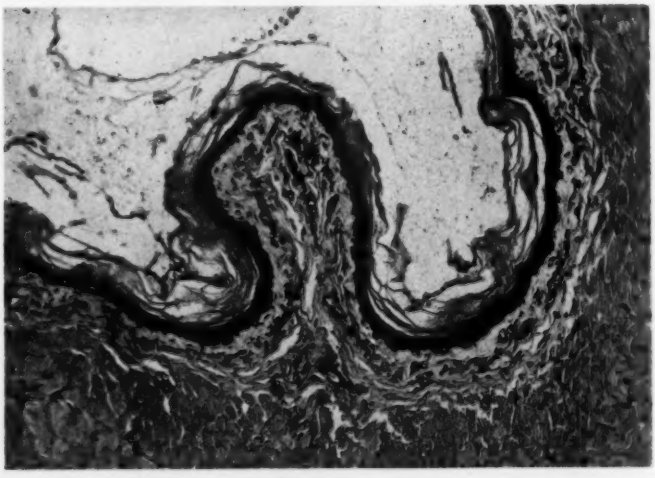

Fig. 2.—Slight submucosal congestion and edma in rat killed four days after receiving 3,000 r of thoracic radiation. Reduced to 63% of mag. × 210.

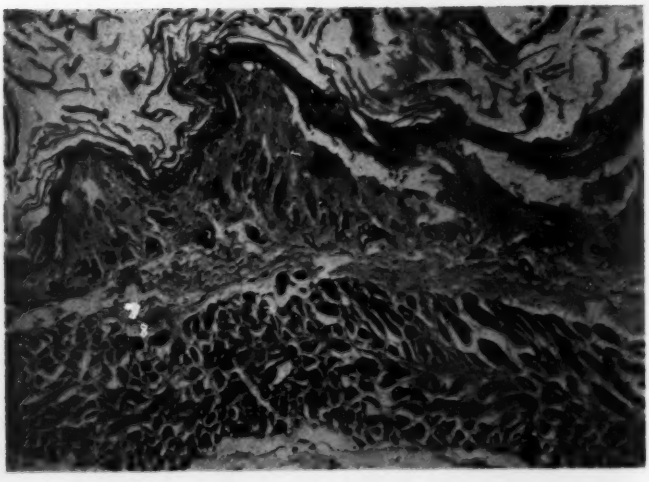

Fig. 3.—Extensive epithelial necrosis in esophagus seven days after thoracic irradiation. Reduced to 63% of mag. × 175.

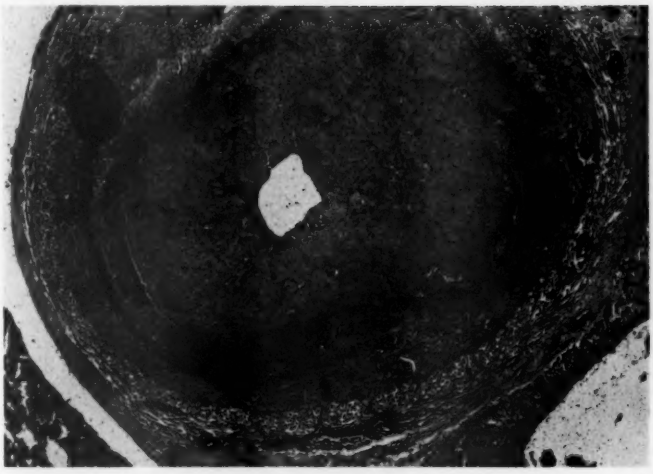

Fig. 4.—Plugging and distention of esophagus with necrotic debris 10 days after irradiation. Note the total degeneration of epithelium with minimal inflammatory response. Reduced to 63% of mag. × 45.

time a few animals showed a moderate submucosal telangiectasia. By the 12th day there was accumulation of inflammatory exudate, in addition to the necrotic debris, in the esophageal lumen, and there was now moderate chronic submucosal inflammation. A few animals showed a margin of healing epithelium at 14 days, when the esophagus was cut longitudinally, though there was still accumulation of necrotic debris within the lumen. By the 16th day many of the animals showed extensive healing, and in a few rats reepithelization of the entire denuded area had occurred. Within this regenerated epithelium there was marked irregularity in the basal layer, and numerous fibroblasts were apparent in the thickened submucosa. Often the regenerated epithelium seemed to show first accumulation of flat, somewhat irregular, basal cells migrating under the necrotic debris. Also there were apparent foci, or islands, of basal cells proliferating under the debris (Fig. 5). This proliferating basal epithelium appeared to dissect between the necrotic debris within the esophageal lumen and the chronically inflamed submucosa. By 20 days most of the animals showed reepithelization of the

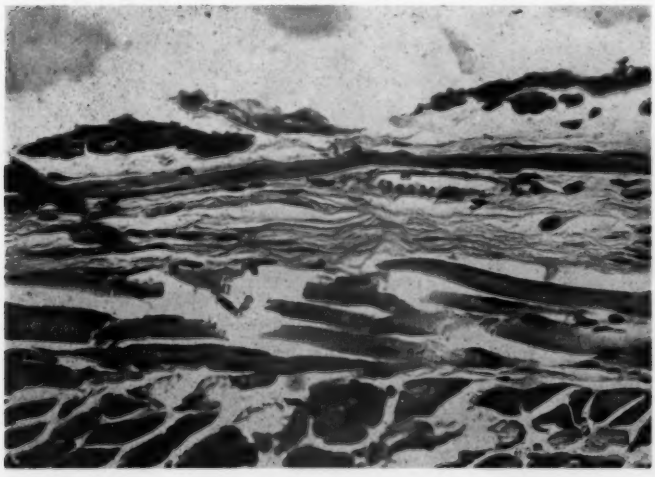

Fig. 5.—Focus of regenerating epithelium, consisting of flattened basal cells. These foci appear during period of rapid healing at about 18 days, between submucosa and necrotic debris. Reduced to 63% of mag. × 510.

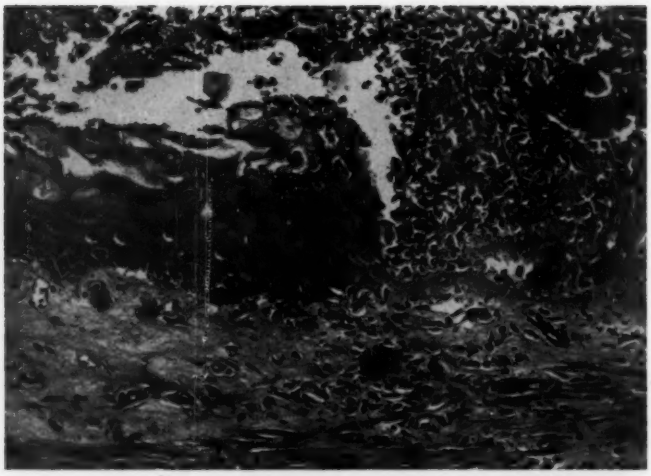

Fig. 6.—Epithelial margin as seen during period of healing. Mitotic figures are common in this advancing epithelial border. Reduced to 63% of mag. × 300.

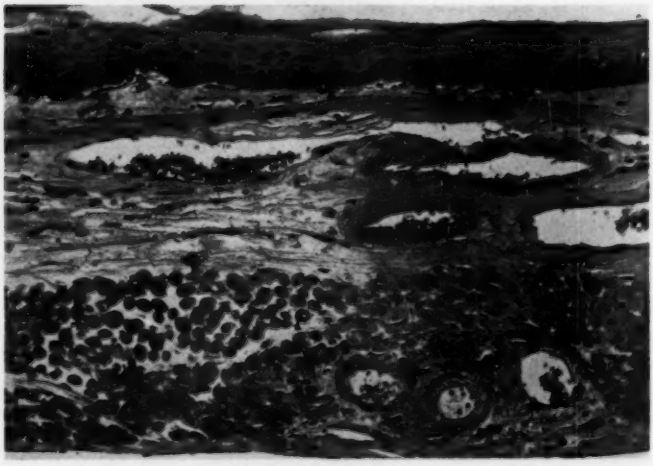

Fig. 7.—During the fourth postirradiation week, animals show restoration of esophageal epithelium, with submucosal fibrosis and telangiectasia. Reduced to 63% of mag. × 260.

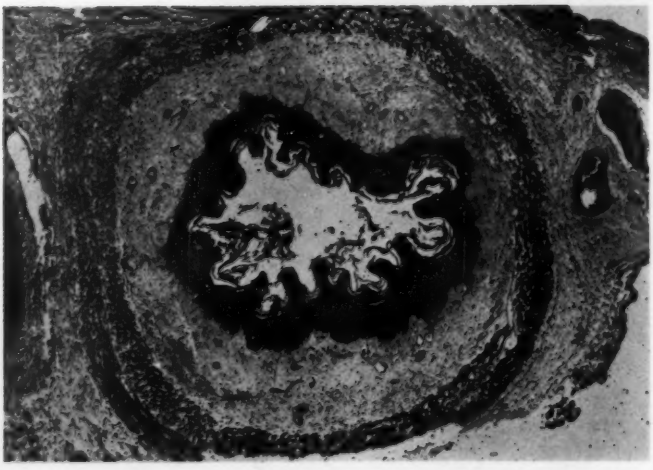

Fig. 8.—Submucosal scarring is conspicuous as a late sequela of radiation esophagitis, as seen six weeks after exposure. Reduced to 63% of mag. × 65.

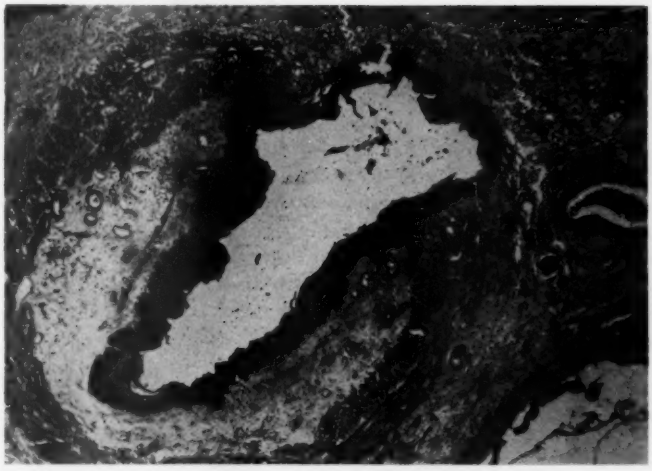

Fig. 9.—Esophageal diverticula with mucosal herniation into a muscularis defect is a common late finding after radiation damage to the esophagus. Reduced to 63% of mag. × 82.

esophagus. Healing appeared to occur both from the margins of the radiation ulcer and, to a slighter degree, from mucosal folds located within the ulcer. The epithelium at the margins of those small ulcers which still persisted at 20 days showed marked lack of organization and appeared to be composed primarily of large, irregular basal cells, with no evidence of the stratification that was seen in the older areas of healing (Fig. 6). Mitoses were common in these margins of epithelium, suggesting rapid proliferation of these cells. All of the animals showed complete restoration of their epithelium by the 25th day (Fig. 7). At this time there was moderate submucosal fibrosis, and this submucosal scarring persisted throughout the remainder of the observation period (Fig. 8). At 30 days, commonly, the epithelium was hyperplastic within the esophagus, and the rete ridges were conspicuous. In animals killed late in the course of the experiment, small esophageal diverticula were conspicuous (Fig. 9). These appeared to be defects within the muscle wall, presumably secondary to inflammation and necrosis within that muscle, with herniation of the esophageal epithelium into the muscularis defect. Three months after exposure the animals showed marked submucosal scarring, with some telangiectasia and moderate atrophy of the esophageal epithelium.

Comment

From these studies it appears that the esophageal epithelium is significantly radiosensitive. In rats exposed to a radiation dose within the range corresponding to that which is commonly used in radiation therapy, there was denudation of the esophagus during the second and third weeks following exposure. These changes confirm and parallel the observations of Engelstad in dogs, though in the present experiment the sequence of degeneration and regeneration was somewhat more rapid than that observed in dogs. These acute changes also parallel to some degree the changes observed by Seaman and Ackerman in humans, and the period of esophageal mucosal erosion in these animals corresponds to the period of dysphagia and substernal burning commonly seen in patients after thoracic radiotherapy.

Healing of the esophageal mucosa occurs fairly rapidly and reepithelization appears to proceed rapidly during the third postirradiation week. Lacassagne[6] felt that reepithelization within the esophagus occurred by epithelial migration from the nonirradiated areas. In this series of animals there was marked proliferation of epithelium from the nonirradiated ends of the esophagus during this healing period, but such migration does not appear to be cap-

able of reepithelizing the entire esophagus in the short period of time during which healing takes place. In addition to this marginal migration of squamous epithelium, foci of squamous cells appeared in the middle portions of the esophagus, commonly in relation to esophageal folds, and these foci showed evidence of marked cell proliferation. Whether these islands of regenerating epithelium represent, or originate from, epithelial cells which have survived the irradiation, or possibly implantation of desquamated cells from the oral cavity, cannot be ascertained.

Submucosal fibrosis is conspicuous in the later stages of healing of radiation esophagitis. During the period of epithelial erosion there is chronic inflammation in the submucosa with some thickening, due both to fibrous tissue proliferation and to edema. With restoration of the epithelium the submucosal fibrosis becomes more striking, and is one of the outstanding features of radiation esophagitis, as mentioned by Seaman and Ackerman. In contrast to the observations of these authors, however, was the minimal change seen in the muscularis in these experimental animals. The scarring which was seen in the muscularis of these animals can probably be explained by the chronic inflammation within the wall of the esophagus during the earlier phases of radiation esophagitis, rather than being a direct effect of radiation on the muscle, as suggested by Seaman and Ackerman. In these animals blood-vessel effects were minimal, and in this observation the findings in the animals parallel those in humans, reported by Seaman and Ackerman.

The development of diverticula from 6 to 12 weeks after irradiation was striking in these experimental animals. Esophageal diverticula secondary to irradiation have been described in humans by Herzog,[7] who felt that such diverticula were due to traction from scar tissue within the mediastinum. In these animals the diverticula appeared to be secondary to focal degeneration of the muscularis, presumably due to inflammation, with secondary herniation of the regenerated epithelium into the submucosal and muscularis defect. Scarring in the mediastinum adjacent to these diverticula appeared to be minimal.

Conclusions

1. Esophageal epithelium is relatively radiosensitive and, in experimental animals with exposures approximating those used in thoracic radiotherapy, shows complete necrosis.

2. Epithelial necrosis, occurring in radiation esophagitis, is seen during the second postirradiation week with healing progressing rapidly during the third week.

3. Submucosal fibrosis and telangiectasia are conspicuous after epithelial restoration and persist as permanent radiation sequelae.

4. Esophageal diverticula, with herniation of mucosa through a defect in the esophageal muscularis, are common late complications of radiation esophagitis.

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Early Pathogenesis of Experimental Histoplasmosis

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The respiratory tract has gained widespread acceptance as the natural portal of entry for the fungal organism Histoplasma capsulatum. Numerous epidemiologic studies have demonstrated that both man and animals probably acquire a primary pulmonary Histoplasma infection from the inhalation of air-borne spores. The spore-producing saprophytic phase of this fungus grows freely in rich organic soils. The conversion of spores to the yeast phase and parasitization of the macrophagic system most logically should occur within the pulmonary parenchyma. From this tissue, yeast organisms would supposedly be easily disseminated hematogenously and overwhelm macrophages within other organs of the reticuloendothelial system, namely, the bone marrow, spleen, liver, lymph nodes, and adrenals. It is the resultant epithelioid-cell granuloma, characterized by marked proliferation of histiocytes filled with parasitized yeast-phase organisms that is the usual pathologic finding described in acute Histoplasma infections.

The respiratory tract as the natural route of infection with H. capsulatum has been verified experimentally. Disseminated histoplasmosis has been produced in mice by direct exposure to contaminated soil [1] and in dogs breathing spore-laden air via tracheal catheter from a culture flask.[2] White mice have been employed most extensively and effectively in the laboratory to produce culturally and pathologically proved Histoplasma infection by various routes. The mycelial phase has served as a very successful inoculum in infecting mice via the intravenous,[3-5] intraperitoneal,[3,6-13] and intranasal [12,13] routes. A relatively few to even a single viable spore has proved capable of causing generalized histoplasmosis in the mouse, resulting in death or chronic infection.[13,14]

Although the pathogenicity of histoplasmosis in the white mouse has been extensively documented, the very early pathologic changes attributed to the presence of the spore have not been described. The immediate response of the host tissue to the mycelial form of H. capsulatum has been demonstrated only at unnatural entry sites.[15-18] The earliest pulmonary pathology in mice infected intranasally with Histoplasma spores was described by Grayston et al.[12] However, the actual presence of the spore, its disintegration and conversion to the yeast phase, and the associated cellular reaction have never been described in the lung of man or experimental animal.

This group of studies was planned primarily to demonstrate such progressive tissue reaction to the Histoplasma spore within the mouse lung immediately following intranasal implantation and its subsequent rupture and conversion to the parasitic yeast phase. Cultural observations concerning the rapidity with which the Histoplasma yeast organisms were propagated and disseminated throughout the reticuloendothelial system of the animal were also made. Death rates were correlated with the intensity of the

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systemic disease. Inoculation of spores directly into the stomachs of mice was performed to distinguish between infection by inhalation and infection by ingestion.

Materials and Methods

Fungus.—The mycelial phase of H. capsulatum, a strain isolated in nature from a silo and productive of large numbers of tuberculate spores, was maintained on simple corn meal agar at room temperature. An isotonic saline suspension of mature tuberculate chlamydospores and mycelial elements was prepared from five- to six-week-old cultures by scraping with a wire loop under sterile saline. Suspensions were concentrated by centrifugation, and the spore density of the suspension was determined in a Levy counting chamber. Viability of the spores was checked by culturing the suspension on corn meal agar. From 60% to 80% of the spores were viable by this method. A suspension was suitable only when clearly identifiable mature tuberculate chlamydospores were sufficiently concentrated to insure absolute detection within pulmonary tissue after intranasal instillation. Variable intranasal doses containing from 5,000 to 20,000 spores in 0.05 to 0.10 cc. of saline were prepared for group inoculations. A suspension of 7,000 to 10,000 spores per dose was used for inoculation directly into the stomach.

Inoculation of Animals.—Approximately 700 female white Swiss mice, each weighing 15 gm., were employed. The saline suspension of mature tuberculate chlamydospores was inoculated intranasally into ether-anesthetized mice. Since the desirable spore count was concentrated in 0.05 to 0.10 cc., 4 to 6 drops of saline suspension from a 25-gauge needle directly into the nares of each mouse served to approximate this infective dose. An additional 60 mice were given an intranasal inoculation of 0.7 to 1.0 cc. of isotonic saline without spores by 25-gauge needle under ether anesthesia.

A slightly curved and blunted 20-gauge needle was passed directly via the esophagus into the stomach for the inoculation of 30 mice. The inoculum was 0.5 cc. of saline containing 7,000 to 10,000 spores. Any animal that appeared to regurgitate the suspension or suffered trauma during the procedure was killed immediately.

Cultures and Pathology.—Of the intranasally infected mice, groups of three to six animals were killed as frequently as at three-hour intervals for pathologic and cultural study over a 10-day period. More extended observations were made on other groups of mice. Large numbers of animals were killed to coincide with the time at which pathology was ascertained. From these, the lungs, liver, spleen, and adrenals were obtained for culture. Each organ was ground separately with antibiotic broth and sterile sand and plated on cysteine-dextrose-enriched blood agar and corn meal agar. Blood cultures, when done, were obtained from the axillary vein. Cultures were made from portions only of the liver and spleen of those animals killed for pathologic study. All mice dying or found dead after infection were cultured, so far as possible, by smearing the cut surface of the lung, liver, and spleen over the media. An autopsy was performed to determine the presence of pulmonary pathology in any animal dying after the intranasal inoculation of isotonic saline without spores.

Multiple microscopic sections were obtained from the various organs comprising the reticuloendothelial system. The lungs were prepared for pathologic study by the intratracheal injection of Zenker's formol solution, and tissue sections were taken from the posterior portions. Hematoxylin-eosin, hematoxylin-eosin-azure, Hotchkiss-McManus, and Gridley techniques were employed in the staining of these sections.

Mortality.—The death rate following intranasal infection with approximately 5,000 spores in another group of 255 mice was observed. The lungs, liver, and spleen from all dead mice were cultured to prove the presence of disseminated histoplasmosis.

Results

Pattern of Organ Dissemination by Culture.—After intranasal inoculation of spores a very consistent pattern of early dissemination of Histoplasma organisms from the lungs to other organs was demonstrated by cultural methods. A rather crude, although quantitative, impression of the increasing profusion of Histoplasma colonies obtained by culture from the respective tissues is shown in the accompanying Table. The lungs, as expected, were consistently positive for the fungal organism from the onset, although increasing numbers of colonies were isolated on subsequent days. By the fourth day, dissemination of the yeast-like organisms from the lungs was apparent from the cultural involvement of the spleen and liver. Progressively heavier growths of H. capsulatum were subsequently obtained from these organs. The adrenal glands became belatedly positive by the 10th day, and the blood stream by the second week, after infection. By the end of two weeks all animals showed heavy and complete reticu-

Organ Dissemination of Histoplasmosis in Mice Infected with Spores by the Intranasal Route *

Days	Organs Cultured				
	Lungs	Spleen	Liver	Adrenal	Blood
1	+	0	0	0	0
2	+ to ++	0	0	0	0
4	++ to +++	0 to +	0 to +	0	0
6	++ to ++++	0 to +++	0 to +++	0	0
8	+++ to ++++	0 to +++	0 to +++	0	0
10	++++	++++	+ to ++++	0 to +	0
14	++++	++++	++ to ++++	++++	0 to ++++
21	++++	++++	++++	++++	0 to ++++
28	++++	++++	++++	++++	0 to ++++

* +=less than 10 colonies; ++, 10 to 25 colonies; +++, 26 to 75 colonies; ++++, too numerous to count.

loendothelial involvement. Although dissemination was obviously occurring by hematogenous spread, the organisms remained relatively scarce to sporadically absent from the blood stream in many animals.

Of the 30 mice infected by inoculation of spores directly into the stomach, only 1 was shown to have acquired disseminated histoplasmosis by cultural screening of the organs of the reticuloendothelial system six weeks after infection. The remaining 29 animals, killed over a six-month period, were found to be culturally negative. Cultures made of colonic contents on all animals were negative for H. capsulatum, regardless of the time interval since infection.

Mortality Rate.—Large numbers of animals infected intranasally with a suspension of 5,000 spores died during the second, third, and fourth weeks after inoculation owing to overwhelming Histoplasma infections, as seen in Figure 1. Of the 255 animals observed for deaths, only 29.4% died within three weeks, and 35.0% died within four weeks, following infection. Cultures of the lungs, liver, and spleen of all dead animals, whenever obtained, were highly positive for H. capsulatum. A second wave of increased mortality in the chronic phase of the disease began during the 11th week after infection and continued through the 16th week. Although there was a subsequent stabilization of the rate thereafter, all of the mice had died within nine months following infection. None of the mice given an intranasal inoculum of isotonic saline without spores died during this nine-month interval, nor did any of the mice infected by inoculation of the stomach with spores die during six months of observation.

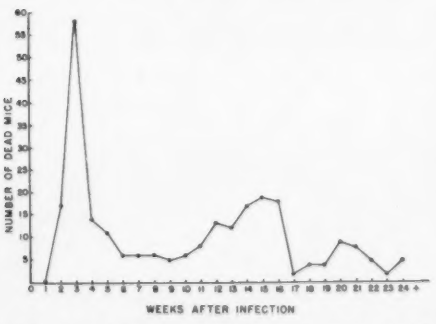


Fig. 1.—Mortality rate of mice inoculated with 5,000 Histoplasma spores intranasally (total 255 mice).

Pathogenesis.—From three to six hours after infection, typical tuberculate chlamydospores were present within bronchioles and alveolar ducts, and large numbers were sufficiently small to have lodged directly within alveoli (Fig. 2*A*, *B*). The cellular reaction to the individual spores either was completely absent or consisted of a few polymorphonuclear leukocytes attached singly to the spore wall (Fig. 2*C*, *D*). The infiltrate about an occasional spore was more cellular and contained a rare round cell (Figs. 3, 4*A*). Tubercles were often elongated, swollen, or globular but remained attached to the spore wall. When fractured, nonviable spores were present, polymorpho-

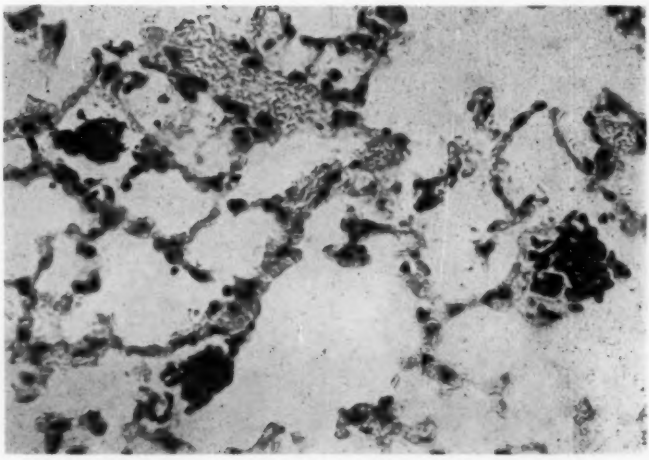


Fig. 3.—Six hours after infection. Typical distribution of spores within alveoli, showing increasing cellular reaction to their presence. Hotchkiss-McManus technique; × 370.

nuclear leukocytic response was more intensive, and white cells had actually invaded the interior of some spores (Fig. 4*B*). Small masses of filamentous elements bearing conidia and the more immature, smooth-walled spores were located in bronchi and

Fig. 2.—Three hours after infection.

A, mature tuberculate chlamydospore resting against the bronchial wall. Note the complete absence of cellular response. Hotchkiss-McManus technique; × 1,000.

B, distinctly tuberculate spore lodged completely in an alveolus without any cellular response. Hotchkiss-McManus technique; × 1,000.

C, two spores in adjacent alveoli with a single polymorphonuclear leukocyte attached to one. The spore on the left has been sectioned in a superficial plane and through many tubercles projecting forward from the wall. Hotchkiss-McManus method; × 1,000.

D, increasing, although minimal, polymorphonuclear leukocytic response to a spore located in an alveolus. Hotchkiss-McManus technique; × 1,000.

Fig. 4.—Six hours after infection.

A, well-preserved tuberculate spore within a bronchiole surrounded by a single row of cells. Hotchkiss-McManus method; × 1,000.

B, fractured, nonviable spore, with more intensive leukocytic response. Hotchkiss-McManus technique; × 1,000.

Fig. 5.—Six to nine hours after infection.

A, mycelial fragments and a single spore within a bronchus. The surrounding cellular reaction is intensive and primarily polymorphonuclear in type. Hotchkiss-McManus technique; × 1.000.

B, distinctly segmented hyphal elements, conidiophores and a single mature tuberculate chlamydospore infiltrated with polymorphonuclear leukocytes within a bronchus. Hotchkiss-McManus technique; × 700.

Fig. 6.—Nine hours following infection.

A, very well-preserved tuberculate spore in an alveolus surrounded by increasing number of polymorphonuclear leukocytes and a few round cells. Hotchkiss-McManus technique; × 1.000.

B, large tuberculate chlamydospore with younger, smooth-walled spore adjoining. Small red globules appear to be dislodged tubercles, partially absent from periphery of adult spore, and small conidiophores. Cellular reaction is slight. Hotchkiss-McManus method; × 1,000.

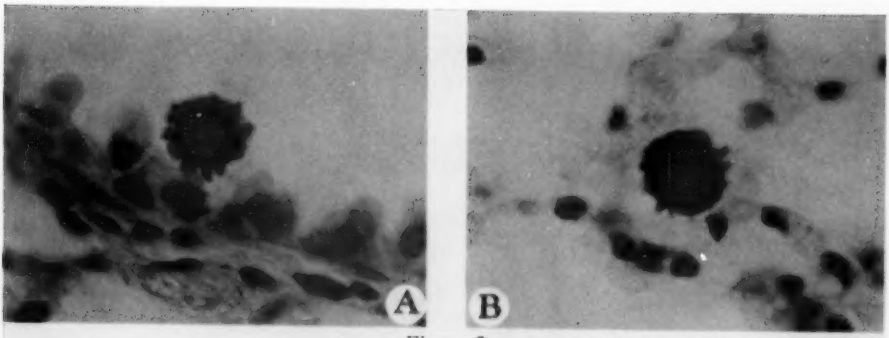
Fig. 8.—Fifteen hours after infection. Cellular reaction to the tuberculate spore remaining focalized and discrete within the alveolus. Particles of disintegration are contained within a few macrophages at the periphery of the exudate and in surrounding alveoli. Some epithelioid cells have begun to replace the leukocytes. Hotchkiss-McManus technique; × 1,000.

Fig. 9.—Twenty-four hours after infection. Diffuse coalescing cellular infiltrates about many intact spores. Note the more characteristic epithelioid and granulomatous appearance of the lesions. Hotchkiss-McManus technique; × 410.

Fig. 10.—Thirty-six hours after infection.

A, extremely well-preserved tuberculate spore surrounded by cellular infiltrate of purely epithelioid nature. Hotchkiss-McManus technique; × 700.

B, focalized mixed cellular exudate within a single alveolus with a few yeast-engorged macrophages in surrounding alveoli. Remnants of a spore cannot be identified. Hematoxylin-eosin-azure stain; × 1,000,



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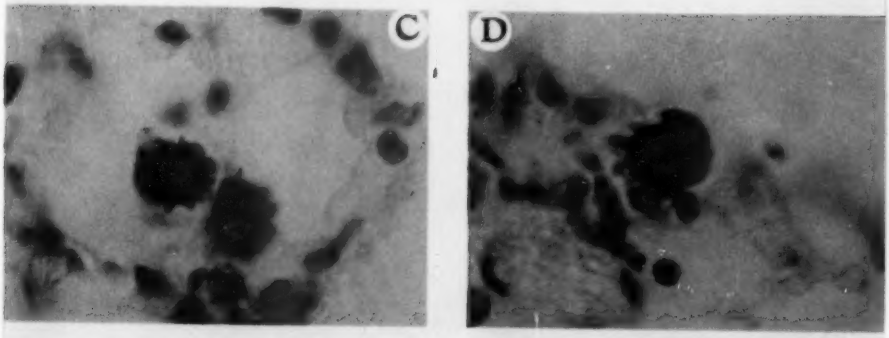

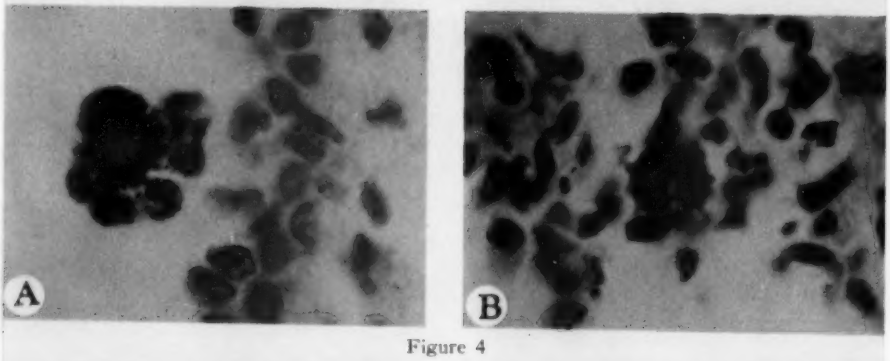

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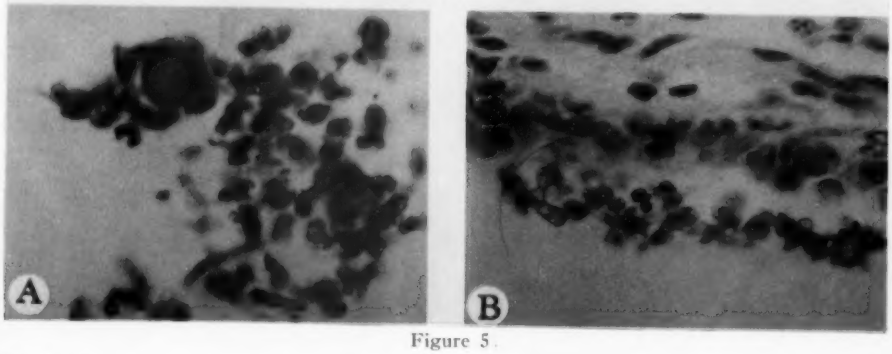

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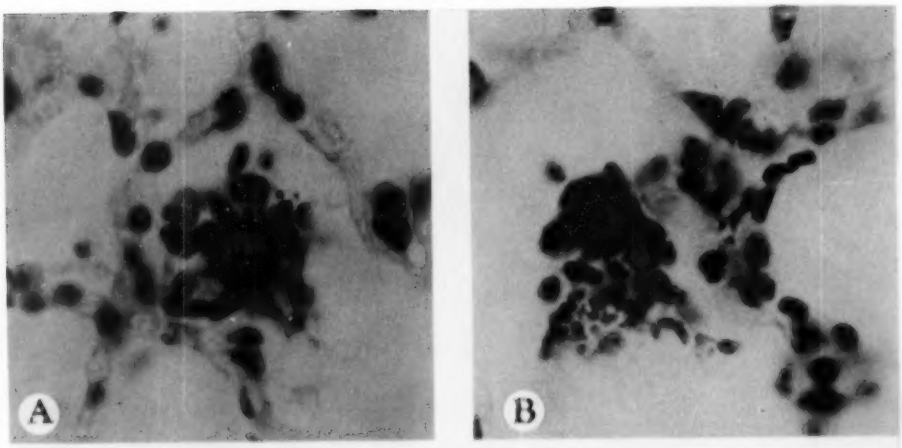

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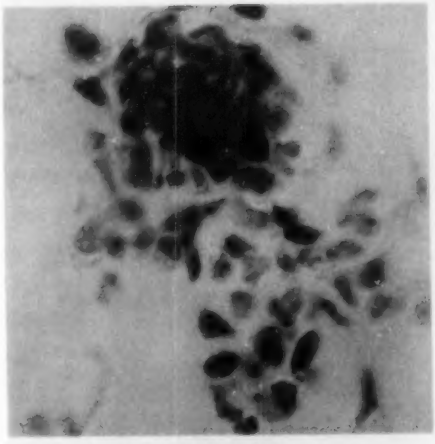

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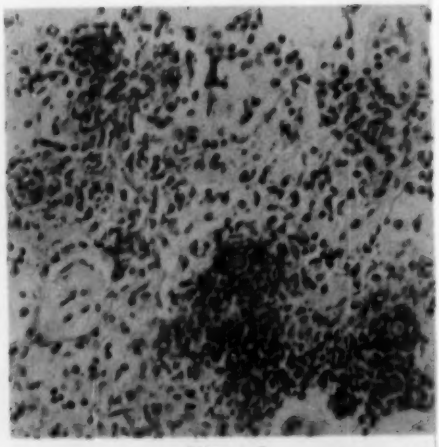

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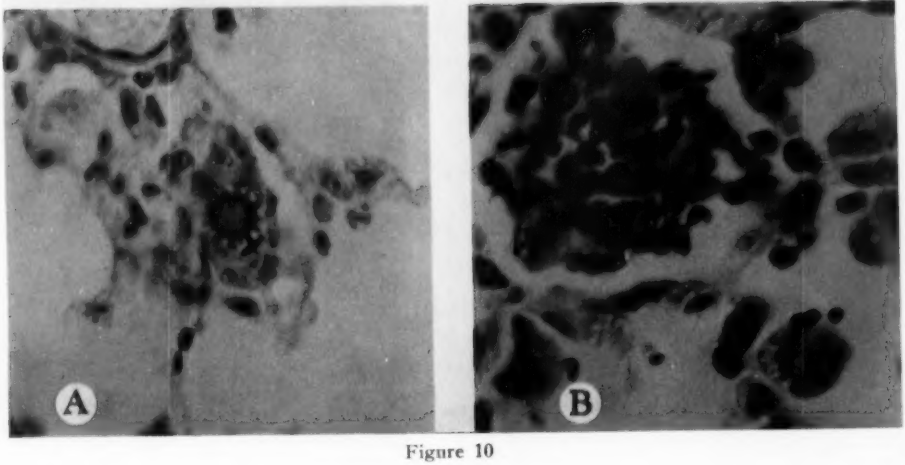

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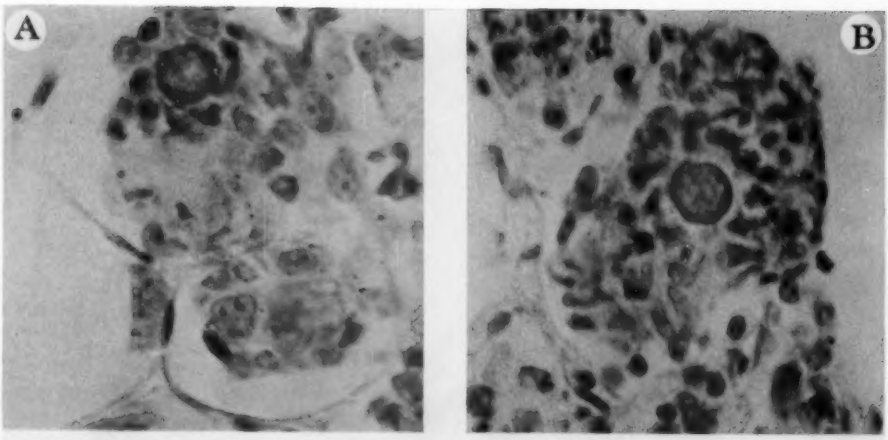

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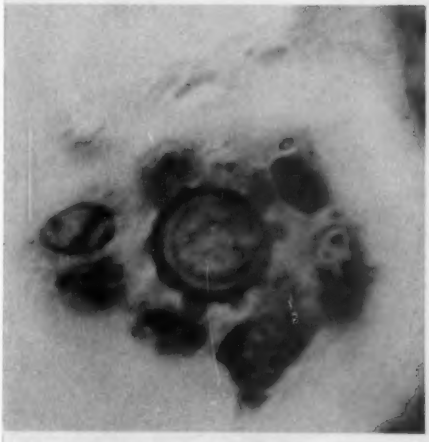

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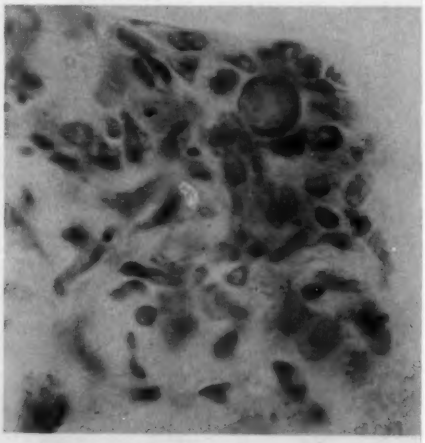

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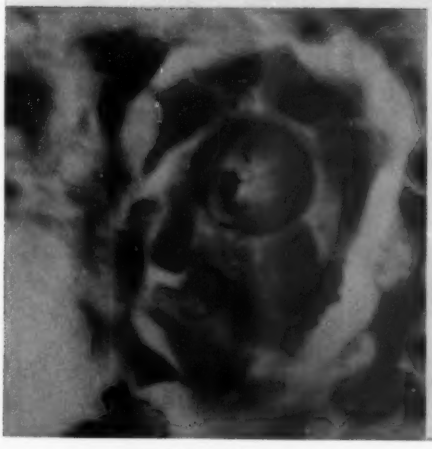

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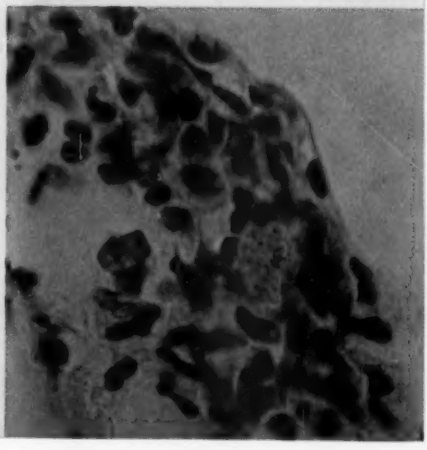

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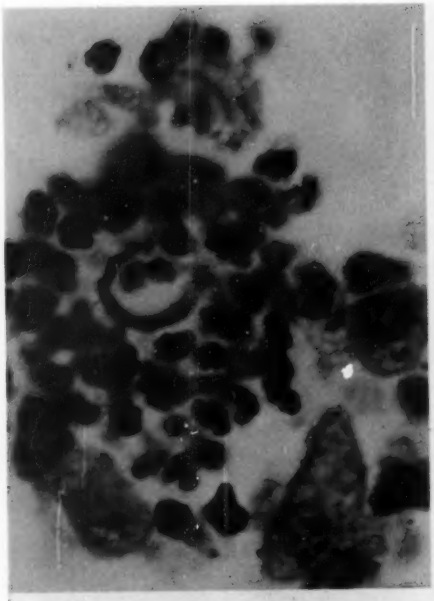

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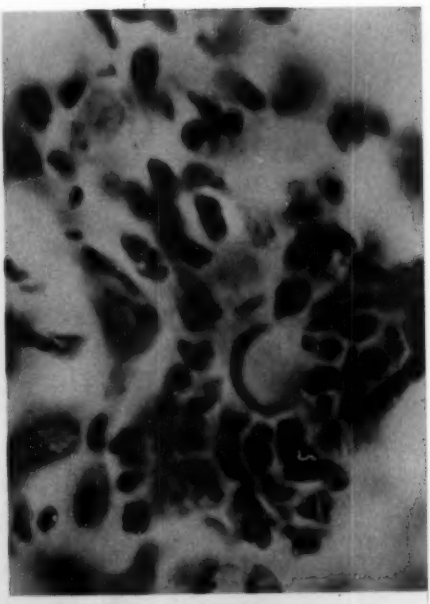

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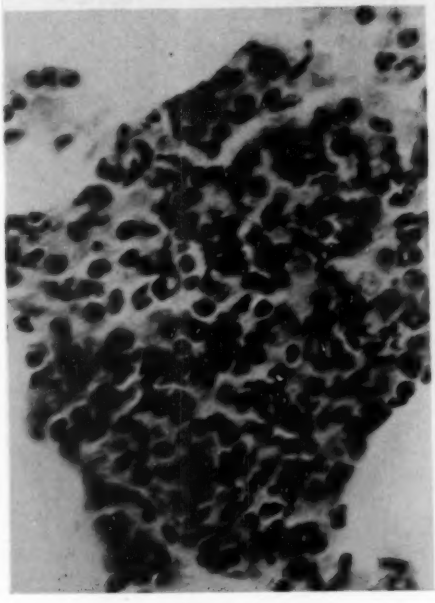

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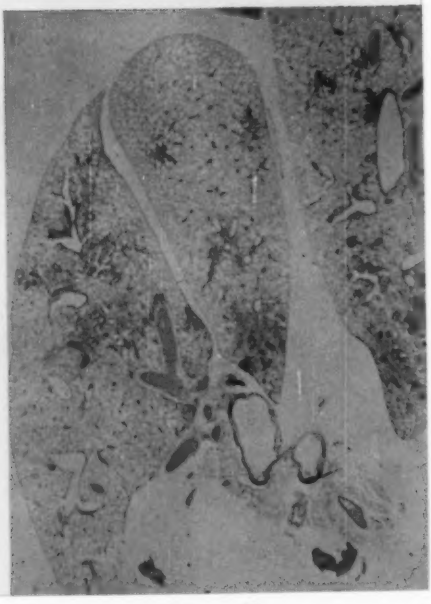

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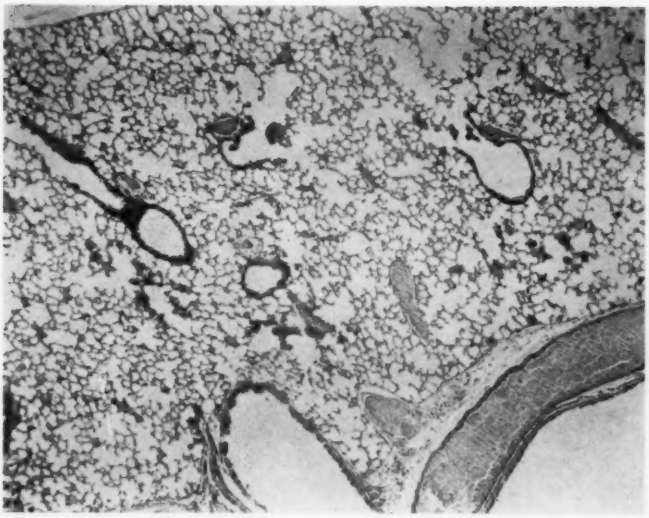

Fig. 7.—Fifteen hours after infection. Typical peribronchial distribution of spores within alveoli. The more prominent spore groupings are easily identified, although the associated infiltrates remain small. Hotchkiss-McManus technique; × 40.

bronchioles (Fig. 5*A*, *B*). The segmented mycelial elements were infiltrated and surrounded with polymorphonuclear leukocytes. The pulmonary tissue in general remained completely free of any other cellular response or edema.

After six hours there was progressive increase in the cellular reaction to the Histoplasma spores. Only conidiophores or the very small smooth-walled spores closely associated with mycelial fragments were infrequently ingested intact by macrophages.

Fig. 11.—Forty-eight hours after infection.
A, epithelioid reaction predominating about an increasingly degenerate spore. Thinning of the wall is apparent, and tubercles have been detached. The exudate has spread into several adjacent alveoli. Hotchkiss-McManus stain; × 1,000.
B, mixed cellular reaction of polymorphonuclear leukocytes and epithelioid cells surrounding a spore now devoid of tubercles and with an obvious defect in its wall. Segmentation within the spore can easily be seen. No yeast cells are identifiable within the macrophages. Hotchkiss-McManus; × 1,000.

Fig. 12.—Sixty hours after infection; epithelioid reaction remaining as a single layer of cells about a thick-walled tuberculate spore. Distinct internal trabeculation and nuclear readjustment can be detected within the spore. Hotchkiss-McManus technique; ×2,000

Fig. 13.—Seventy-eight hours after infection. Smooth-walled spore with obvious defect in wall, through which there are bulging and pouring forth of inner contents. Chitinous fragments and a few yeast cells are present within cells of the surrounding infiltrate. Hotchkiss-McManus technique; × 1,000.

Fig. 14.—One hundred two hours after infection. Ruptured empty spore in an alveolus with exuded contents phagocytosed by adjacent macrophages. Hotchkiss-McManus method; × 1,850.

Fig. 15.—Seventy-two hours after infection. Distinct globular mass of Histoplasma yeast cells surrounded by epithelioid cells in a location similar to that previously occupied by a spore. Hotchkiss-McManus technique; × 700.

Fig. 16.—Seventy-two hours after infection. Polymorphonuclear response predominating in area about a disintegrating empty spore. The missing fragment of the wall has been engulfed by phagocytes, and large macrophages contain yeast cells released from the spore. Several polymorphonuclear cells have invaded the spore wall. Hematoxylin-eosin-azure stain; × 1,350.

Fig. 17.—Eight days after infection. Rare persisting fragment of spore wall within round-cell infiltrate being destroyed. Hotchkiss-McManus; × 1,400.

Fig. 18.—Seven days after infection. Typical granuloma of densely cellular infiltrates composed of lymphocytes and epithelioid elements. Yeast cells are difficult to identify. Hematoxylin-eosin stain; × 700.

Fig. 19.—Ten days after infection. Large, coalescing exudates extensively involving pulmonary tissue, although located peribronchially, where spores originally lodged. Hematoxylin-eosin; low-power mag.

By nine hours after infection, the leukocytes completely surrounded the spores and wedged themselves deeply between the tubercles (Fig. 6*A*). Tubercles about the periphery of some spores were apparently being slowly dislodged by the degenerative forces at work (Fig. 6*B*). The Histoplasma spores, becoming increasingly marked by exudative response, were most typically distributed within the alveoli and concentrated peribronchially (Fig. 7).

Within 15 hours, although the infiltrates remained principally leukocytic, epithelioid cells had become increasingly prominent (Fig. 8). Hyphal segments and immature spores were no longer discernible. Yeast forms were still not in evidence. More eosinophilic particles arising from spore disintegration were noted in wandering macrophages.

By 24 hours after infection the inflammatory reaction to the still intact tuberculate spores had increased sufficiently to break from the confines of the alveoli containing them (Fig. 9). The exudates involved increasing numbers of neighboring alveoli and began to assume a granulomatous appearance. Spores remaining in bronchi or bronchioles had initiated a profuse leukocytic response about them and were thus secured against the epithelial lining of the wall.

By 36 hours following inoculation, most, if not all, polymorphonuclear leukocytes had degenerated and were replaced by epithelioid cells (Fig. 10*A*). Many spores, although thickly engulfed by exudate, remained extremely well-rounded, thick-walled, and with tubercles intact. The exudate continued to fill alveoli adjacent to the spores, and macrophages positioned themselves more peripherally. A very few of these macrophages, for the first time, contained some yeast forms of H. capsulatum (Fig. 10*B*). The implication was that not only hyphal elements, conidiophores, and small, smooth spores but a few larger spores had been transformed into small numbers of yeast parasites as early as this 36-hour period following infection. No spore fragments were to be seen within any of the infiltrates, however.

From 48 to 60 hours, the spores had begun to develop a pattern of internal trabeculation. There was a gradual progression and increasing distinctness in this internal segmentation, with clumping of the nuclear material in the majority of the spores from that time onward (Figs. 11*A*, *B*, and 12). It appeared as though yeast conglomerates were taking form within the spores. Concentration of the nuclear material occurred with retraction from the spore wall. The disintegration of the spores began obviously to get under way, as characterized by loss of tubercles and thinning of the wall, to frank breaks or overlapping in the wall (Fig. 11*A*, *B*). A thick collar of epithelioid cells had almost completely replaced the leukocytic infiltrate about the tuberculate spores. In some instances, a single layer of epithelioid cells had neatly arranged itself about the periphery of an internally trabeculated spore within an alveolus, in the absence of any further cellular response (Fig. 12). Generally, the infiltrates had continued to increase in size and involve additional contiguous alveoli. A few groups of intact spores with minimal surrounding cellular response could still be seen within bronchioles. A few of the chitinous capsules of mature spores maintained their integrity, and the tubercles remained prominent peripherally. There was still no evidence that any mature spores had been engulfed *in toto* by single macrophages, nor had any multinucleated giant cells made an appearance. Yeast forms remained significantly absent or rarely present within the few macrophages which were becoming more prominent as components of the cellular reaction to the spore. The continuing, although gradually degenerating, integrity of the spore wall was maintained.

The most crucial phase in spore disintegration had occurred by 72 hours after infection. During the fourth and fifth days after inoculation, all of the discretely tuberculated spores lost their projections and

became smooth. Progressive thinning of the chitinous spore walls resulted in many more obvious defects and actual fractures, with displacement of segments. Bulging and exuding of the spore content occurred through these rents in the wall (Figs. 13 and 14). Eosinophilic debris, suggestive of dislodged tubercles and chitinous fragments, were components of the infiltrate surrounding the spore (Fig. 13). Many of the capsules which showed frank rupture appeared empty, and the exuded contents had been phagocytosed by the macrophages in the vicinity. An occasional globular mass of Histoplasma yeast cells remained free, although compactly contained by epithelioid cells in a location comparable to that assumed by spores earlier in the course of the infection (Fig. 15). It appeared as though the entire yeast content of a spore had been disengorged at the site.

As disintegration of the empty spores continued, the polymorphonuclear leukocyte often comprised the inner ring of cells about the spore (Fig. 16). There was frank invasion of the larger remnants of spore walls by these leukocytes. Portions of the wall were phagocytosed by macrophages and carried away from the immediate environment. At the very periphery, and remaining quite free of the exudate immediately surrounding the disintegrating spores, macrophages were engorged with numerous yeast cells, undoubtedly released from the doomed spore. Free macrophages were becoming more diffusely scattered in alveoli quite distant to the focal infiltrates. Proliferation of the yeast organisms within the large macrophages was more obvious. No giant cells had made their appearance. Only a very few spores persisted beyond five days and showed variable degrees of disintegration. There was a rare, although surprising, intact tuberculate spore that would remain unscathed and free of surrounding exudate.

Beyond one week after infection only a few remnants of spore walls were still in the process of being destroyed (Fig. 17). By 10 days after infection, the pathologic picture had finally converted to scattered granulomatous infiltrates, described in the literature as typical for histoplasmosis. The infiltrates remained focal but involved large numbers of alveoli (Fig. 18). The cells comprising the exudate were primarily mononuclear, with a few polymorphonuclear cells interspersed. Macrophages containing yeast organisms were scattered sparsely

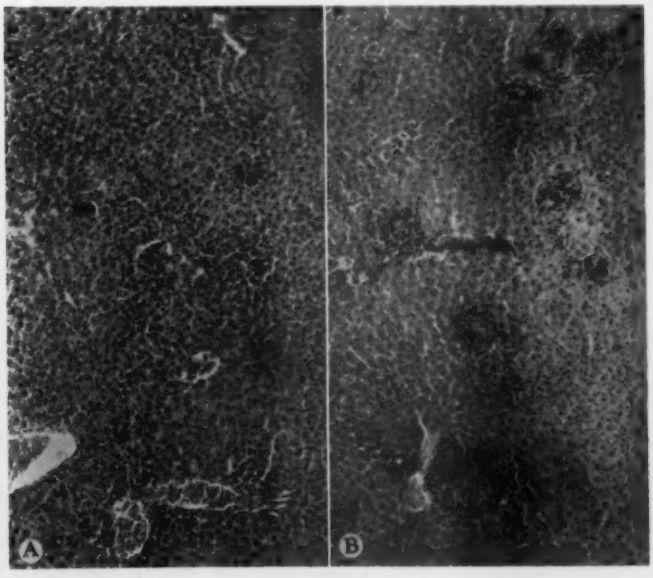

Fig. 20.—Dissemination of infection to liver.

A, six days after infection—very minute round-cell infiltrates scattered sparsely throughout the liver. Hematoxylin-eosin stain; × 80.

B, 18 days after infection. Numerous and enlarged typical granulomatous infiltrates. Yeast organisms are impossible to identify within these lesions. Hematoxylin-eosin stain; × 80.

among these cells. There was a semblance of preservation of tissue architecture, since the exudative cells maintained a loose association. No tissue destruction, consolidation, or edema resulted. Giant cells were never seen at any stage in the progressive development of these early granulomas. Diffuse pulmonary histoplasmosis had developed by 10 days after infection, the exudative foci being concentrated peribronchially and located where spores had previously been seen to initiate a tissue response (Fig. 19).

The extrapulmonary dissemination of the yeast organisms was apparent pathologically in the liver and spleen by the sixth day following infection (Fig. 20*A*). Very minute and localized round-cell infiltrates were observed scattered scantily throughout the liver parenchyma. These lesions increased in size and numbers within a few weeks, maturing to the more typical granulomas, comprised of epithelioid cells centrally and lymphocytes peripherally (Fig. 20*B*). Macrophages containing yeast organisms were almost impossible to find within these infiltrates.

Comment

These studies have demonstrated culturally that the dissemination of yeast organisms began within the lung parenchyma during the first few days following intranasal instillation of tuberculate Histoplasma spores and mycelial elements. The yeast forms probably proliferated almost immediately, arising at first from some small conidiophores and hyphal fragments accompanying the mature spores. The number of yeast organisms was undoubtedly too small and dispersed to be detected in the tissue sections during the first few days after infection.

The progressive conversion of spores to yeast-like cells and the proliferation of histiocytes with multiplication of yeast cells within them, satisfactorily explains the spread of the infection to the liver and spleen by the fourth day and the subsequent heavier growth patterns of Histoplasma organisms from these tissues. Dissemination via the blood stream must be assumed to have actively occurred at that time. However, the blood cultures showed great variability in the numbers of Histoplasma organisms are released in showers or are organisms present. This suggests that the sufficiently diluted at times so as to make the chance for positive cultures quite unlikely.

By the end of the second week, extensive involvement of all organs of the reticuloendothelial system had occurred, and the number of organisms recovered from these tissues by culture was large and remained stationary. The growth pattern of the Histoplasma fungus in the lungs, spleen, and liver reached its maximum within a week following the first appearance of the organism by culture. Such an exponential growth of Histoplasma in tissues for 7 to 14 days following infection with yeast organisms intravenously has been previously reported.[19] The initiation of the seven-day period of marked multiplication of yeast in the spleen and liver, demonstrated in our studies, also followed exactly that interval of approximately 72 hours required for spores to disintegrate and release yeast organisms.

Direct correlation could be made between the high mortality rate occurring acutely during the second, third, and fourth weeks of infection and the time at which generalized dissemination had reached its peak by cultural proof. Tissue sections of the infected mouse lung showed only rare intact spores persisting after one week after infection. This indicated that the yeasts had been discharged into the tissue, had disseminated, multiplied, and subsequently overwhelmed the reticuloendothelial defenses, resulting in death for many animals. After this acute onslaught, the death rate stabilized, to be followed by a steady increase in mortality from the 11th through the 16th week following infection. These more chronic deaths probably resulted from increasing debilitation and were similar to those observed by others.[13]

Certainly, the gastrointestinal route did not serve as a significant portal of entry for infection with H. capsulatum spores. The single animal positively infected by this route might even be assumed to have incurred its disease by aspiration or traumatic injury to the stomach mucosa.

Because the theory of the air-borne route of infection by H. capsulatum implies that spores must be sufficiently small to penetrate into the alveoli, these studies explicitly prove that such penetration can easily be achieved. It has been shown by others that the great majority of tuberculate and nontuberculate spores of 14 strains of H. capsulatum, whether cultured for one or five months, remained less than 5μ in diameter.[20] Particles which are larger are believed rarely able to penetrate the alveoli. It has also been demonstrated that characteristic mature tuberculate chlamydospores were produced in small numbers by these 14 strains (2.4% of all spores were tuberculate at one month and 6.7% at five months), but that this production varied from strain to strain. The Histoplasma strain used in the present studies has been notable for its ability to produce many viable tuberculate spores on artificial medium within six weeks. Measurements of representative tuberculate mature spores proved that the majority were 5μ or less in size. Such mature spores encountered no difficulty whatsoever in penetrating deeply into the alveolar ducts, atria, and alveoli proper. A few, probably the spores larger than 5μ, lodged in the larger air passages of the lungs. Therefore, the conclusion by Cozad and Furcolow[20] that only small, nontuberculate Histoplasma spores are the infecting agent, rather than the larger, tuberculate chlamydospore, and that the latter serve only to perpetuate the organism through unfavorable conditions, can no longer be entertained. The small, smooth spores and mycelial elements are undoubtedly capable of producing yeast organisms, but this study specifically documents the conversion of the typically mature tuberculate spore to the parasitic yeast form within pulmonary parenchyma. Because it is also the more capable of remaining viable under unfavorable conditions in nature, the mature spore is even more logical as the primary agent of infection.

The early tissue reactions to an inoculum of Histoplasma chlamydospores in the rabbit's eye,[15] in guinea pigs subcutaneously,[16] and in the ear chamber of rabbits[18] have been characterized by initial leukocytic, and subsequent epithelioid, infiltration. Multinucleate giant-cell proliferation resulted, and other giant macrophages phagocytosed intact spores in the first few days. The degenerative change occurring within the chlamydospores during the first week of infection was quite comparable to that observed in the mouse lung. However, multinucleate giant cells never made an appearance, nor did macrophages engulf large intact spores in lung tissue. The response of these extrapulmonary tissues would be expected to differ from lung tissues on the basis of variations in histology, vascularity, and temperature.

It may be concluded that the inhaled Histoplasma spore goes through comparable phases in the lungs of man and that early pulmonary infiltrates of histoplasmosis are thus established. The roentgenographic distribution of the diffuse pulmonary infiltrates resulting from heavy epidemic exposure in humans compares most favorably with the extensive involvement seen in the mouse lung during the second week following experimental exposure. The respiratory tract serves as the only logical route of infection with H. capsulatum in man or animal.

Summary

The early pathogenesis of experimental histoplasmosis in mice infected intranasally with mature tuberculate spores of Histoplasma capsulatum is described. The inhaled tuberculate spore is proved responsible for diffuse areas of pulmonary exudate, the formation and release of yeast-like organisms, and the subsequent dissemination of these organisms to other reticuloendothelial tissues. Within 10 days after infection

this process has been completed, and the typical granuloma of histoplasmosis is established. Mortality rates are correlated with the stages in pathogenesis.

We wish to express our sincere gratitude to Dr. Eleanor M. Humphreys, Professor Emeritus, Department of Pathology, The University of Chicago School of Medicine, for her sound advice and encouragement during these studies.

Department of Medicine, The University of Chicago (Dr. Procknow).

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Pulmonary Arteritis, Glomerulonephritis, and Congenital Heart Disease

An Unusual Triad

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Lesions of periarteritis nodosa confined to the pulmonary arterial tree are unusual, but have been reported in a number of patients suffering from pulmonary hypertension due to various causes.[1-9] The association of acute pulmonary arteritis with acute glomerulonephritis and congenital heart disease is much rarer, and we have found reference to only one similar case.[1] It is of interest that in both the present case and that of Kipkie and Johnson [1] the disease of lungs and kidneys made their appearance and progressed to fatality at the same prepuberal age of 11 years.

Report of a Case

Clinical History

The patient was an 11-year-old white mongoloid girl at the time of her final hospital admission. Her previous admissions numbered eight, all during the first two winters of life, for respiratory infections, which were treated on each occasion with penicillin. Tonsillectomy had been done at the age of one year. From the age of 2 years until a week before her final illness she had been in apparent good health.

The terminal illness began with cough, shortness of breath, and occasional vomiting. She was at first considered to have bronchopneumonia and was treated with penicillin, and later with a sulfonamide, which is not further identified in the outpatient history.

At the time of admission to Hotel Dieu Hospital she was very short of breath and exhibited cyanosis and periorbital edema. Auscultation revealed dullness and crepitations at both lung bases. The blood pressure was 134/70 mm. Hg; the pulse was 88 per minute and regular. The oral temperature was 99.4 F. A harsh apical systolic murmur was heard over the precordium. There was diffuse reddening of the pharyngeal mucosa.

Urinalysis revealed a specific gravity of 1.012 and 4+ proteinuria, with 20 red blood cells and 2 white blood cells per high-power field in the centrifuged urinary sediment. Hemoglobin was 12.4 gm/100 cc., and white blood cells numbered 17,950 per cubic millimeter.

During the week in which she was in hospital, before death, she became incontinent of urine, after a recorded output of 250 cc. during the first 24 hours. Her temperature rose gradually to 102 F and fell to normal levels during the last two days. After a single dose of Dicrysticin (procaine penicillin G with buffered penicillin G sodium and streptomycin and crystalline dihydrostreptomycin sulfates), she was treated for the remainder of her illness with chloramphenicol and digitalis. She failed to improve and died seven days after admission.

Autopsy Findings

Apart from the obvious deformities of mongolism, the main findings were in the heart, lungs, and kidneys. In the heart there was a circular common atrioventricular septal defect 2 cm. in diameter,

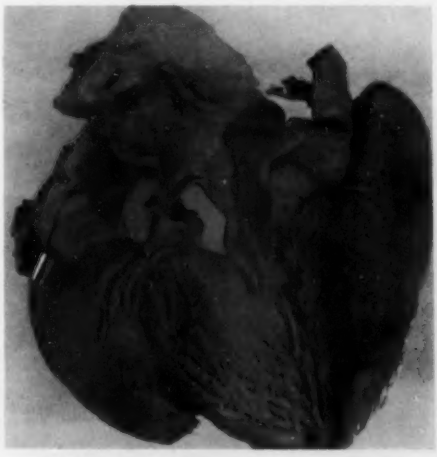

Figure 1

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From the Departments of Pathology, Hotel Dieu Hospital and Queen's University Faculty of Medicine.

with mitral and tricuspid valves continuous with one another through the defect (Fig. 1). The heart weighed 278 gm. The most obvious hypertrophy was in the musculature of the right ventricle, which measured 1.3 cm. in thickness close to the A.V. ring. The right and left lungs weighed 300 and 250 gm., respectively, and there was bilateral anomalous superlobation. They were of moderately increased firmness, and on section the blood vessels were considered to be unusually prominent and thick-walled. The peribronchial parenchyma was indurated and dark red.

The kidneys weighed 136 and 127 gm. Each was diffusely enlarged, and the cortical surfaces were pale and spotted with tiny, pinpoint hemorrhages in classical, flea-bitten fashion. On section, cortical pallor was obvious, and the small hemorrhages tended to assume a streaky, linear configuration. The renal blood vessels were not grossly abnormal. The only other positive finding in the autopsy was the presence of many prominent, soft, pink lymph nodes, 1 to 3 cm. in diameter, in the mesentery and mediastinum.

Microscopic Findings

Microscopic examination of the lungs revealed widespread, necrotizing arteritis affecting the medium-sized and small branches of the pulmonary artery in all lobes. An irregular distribution of these lesions was indicated by the finding of adjacent uninvolved and damaged segments. Lesions seemed particularly frequent at points of bifurcation when serial section studies were made. All pulmonary arterial lesions appeared to be of about the same age. They were characterized by swelling, necrosis, and fibrinoid alteration of the intima and media, with frequent extension of the process to involve the adventitia and adjacent lung parenchyma (Fig. 2). There was a heavy cellular infiltrate, consisting mainly of neutrophil leukocytes, but with considerable numbers of eosinophils. Histiocytes, plasma cells, and lymphocytes were present in small numbers. Several of the involved vessels showed aneurysmal dilatation and partially occlusive thrombosis. Arteritis was not found in vessels of any of the other abdominal or thoracic viscera or in the brain.

Figure 3

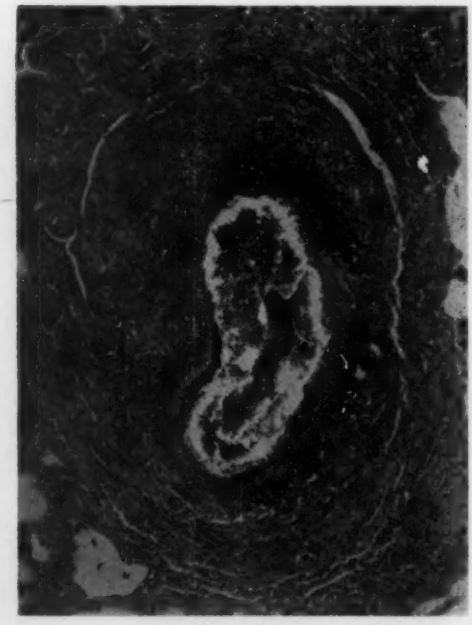

Figure 2

The renal changes were those of acute diffuse glomerulonephritis. There was generalized hypercellularity of glomeruli with reduction in blood content of the capillaries (Fig. 3). Both endothelial and epithelial

cells of the glomeruli were numerically increased. Occasional adhesions between glomerular lobules were seen, as were glomerulocapsular synechiae and epithelial crescents. The cortical tubules were all dilated. Some contained erythrocytes; in others there were homogeneous acidophilic casts or collections of neutrophil leukocytes. Fine basal vacuoles in the epithelial cells of some of the proximal tubules reacted positively with fat stains. The interstitial tissue was edematous, and contained lymphocytes. The intrarenal vessels were not abnormal.

Sections of the enlarged lymph nodes showed only nonspecific changes of generalized edema and widening of sinusoids. Sections of brain, lungs, liver, spleen, reproductive organs, and endocrine viscera were interpreted as normal.

Comment

The association of congenital heart disease, pulmonary arteritis, and acute glomerulonephritis appears to have been reported only once before.[1] The case reported by Kipkie and Johnson [1] is of special interest in that the patient also was an 11-year-old child, though a boy, whereas the present patient was female. While the identity of age of the two cases might well be fortuitous, it is interesting to speculate that the endocrine awakening of puberty may have had pathogenic importance in each case.

The relationship between arteritis confined to the pulmonary circuit and pulmonary hypertension has been much more frequently noted, and we have found reference to 18 such cases (Table). These cases cover a wide range of ages (0 to 47 years).

There has been recent experimental evidence suggesting that fibrinoid necrosis may result from elevation of luminal pressure in the absence of other causes.[12] While there is ample evidence that periarteritis of this type is usually a result of hypersensitivity,[10,11] it can be suggested from the foregoing that the localization of the arterial lesions is determined by abnormally high pressures in the vessels that develop lesions.

Although we have no direct evidence of hypersensitivity in the present case, a sequence of pathogenic events may be suggested to explain partially the association of lesions in this case. This patient, and that of Kipkie and Johnson, had pulmonary hypertension due to septal defects. In addition, they were probably experiencing the physiological rise of blood pressure that accompanies puberty.[13] This may then have become exaggerated during an attack of acute glomerulonephritis, much of the excess blood pressure being transmitted to the pulmonary circuit via the large septal defect. Into this combination of events an arteritis-inducing immune reaction was introduced, and the lesions became localized in the pulmonary arterial tree, already somewhat

Cardiovascular Lesions Associated with Pulmonary Periarteritis Nodosa: Eighteen Cases

No. of Cases	Cardiovascular Lesion	Age of Patients, Yr.	Author
2	Eisenmenger complex	11	Kipkie & Johnson[1]
		11	Old & Russell[5,6]
1	Cor triloculare	3½	Braunstein[3]
3	Patient ductus Botalli	7	Hultgren[4]
4	Pulmonary hypertension	7	Braunstein[3]
		16	Braunstein
		0	Elwood[5]
		2	Symmers[6]
4	Cor pulmonale	10	McKeown[7]
		13	McKeown
		24	McKeown
		42	McKeown
4	Mitral stenosis	28	Braunstein[3]
		16	Braunstein
		47	Braunstein
		2	Weitzmann & Husain[8]

damaged by its high and rising pressure. It might even be speculated that the antigenic stimuli for both the nephritis and the arteritis was the same, since both lesions appear of comparable age.

It is to be hoped that further cases of this unusual triad will be reported, and that from them more may be learned of the pathogenic factors involved.

Summary

A case of apparently simultaneous acute glomerulonephritis and periarteritis of the pulmonary arterial tree in a mongoloid child with atrioventricularis communis is reported. The role of pulmonary hypertension in the pathogenesis of the arteritis is discussed.

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Cortisone and Radiation

III. Histopathology of the Effect of Cortisone on the Irradiated Rat Kidney

LIEUT. COL. CHARLES C. BERDJIS (MC), U.S. Army

Introduction

In the previous studies,[1,2] it was shown that cortisone may modify the course of histopathologic reaction of lung parenchyma of irradiated rats. It was pointed out that cortisone administration in irradiated lungs affects the vascular channel by producing capillary thrombosis, increased capillary permeability, and diffuse or petechial hemorrhages. It was concluded that cortisone may be responsible for vascular disorders frequently encountered in the irradiated rat lungs.

The earlier reports[3-7] provide the data on glomerular lesions in rabbits given cortisone. Although Wilens and Stumpf[7] stated that cortisone administration failed to produce similar lesions in the other species, including man, we found that kidneys of Sprague-Dawley rats showed evidence of marked damage by cortisone.

The present study was conducted to ascertain the effects of cortisone in kidney both in irradiated and in nonirradiated cortisone-treated rats.

Material and Methods

Four groups of 6-month-old healthy Sprague-Dawley rats were used in this experiment. They were distributed as follows:

Group I	Control, untreated	30
Group II	Irradiated without any other treatment	30
Group III	Irradiated and cortisone-treated	30
Group IV	Cortisone-treated only	30

Sixty animals from Groups II and III received 3×500 r to both kidneys. The conditions of irradiation were as follows: 250 kv. machine; 15 ma.; 100 cm. target distance; filters Cu-0.5 mm. and Al-1 mm.; rate 65 r/min. The rats received irradiation under anesthesia (pentobarbital [Nembutal] sodium, 34 mg/kg. for males and 32 mg/kg. for females) to both kidneys, and partial-body x-irradiation, 500 r in each exposure.

The rats of Groups III and IV received 3 mg. of cortisone acetate daily by intramuscular injection for a period of 100 days.

The rats of all groups were kept under observation and fed with standard laboratory diet for a period of approximately 100 days. Except for a few animals that died during the experiments, all others were killed at the end of 100 days.

The entire genitourinary organs of the rats were carefully dissected and fixed in 10% formalin. They were embedded in paraffin, sectioned, and stained with the routine hematoxylin-eosin method unless special mention was made.

Results

The following data are related to the changes which occurred in the kidneys as a result of cortisone administration and irradiation, simultaneously or separately. The changes observed in the gonads will be reported elsewhere.

1. *Controls.*—No gross or histologic changes were observed in the kidneys of control, untreated animals * (Fig. 1*A*).

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* Although the structure of the normal rat kidney differs in many respects from that of a human kidney (Sternberg et al.[8]), it is interesting to note that the structure of the glomeruli, except for a certain degree of sexual dimorphism, is similar to that of man. The sexual dimorphism in the mouse, described by Crabtree,[9] and much less apparent in the rat, consists of a peculiar difference in the structure of Bowman's capsule. The latter is composed mostly of tall or cuboidal epithelial cells in the male and flattened epithelial cells in the female. This observation is of some value because irradiation changes this sexual dimorphism so that the male and female kidneys are much alike (Berdjis and his co-workers[10-12]).

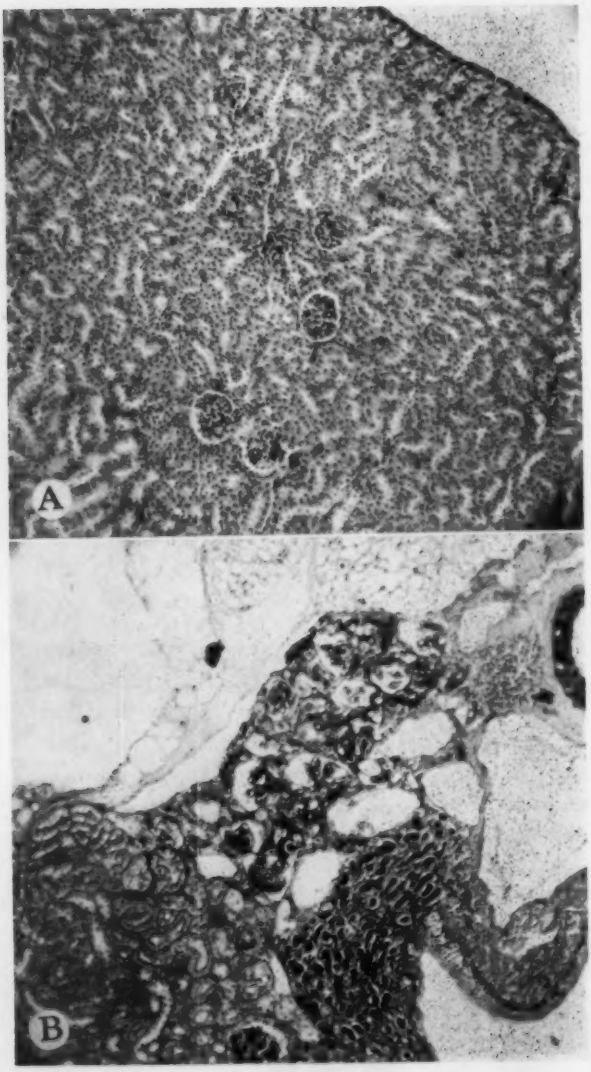

Fig. 1.—*A*, photomicrographic section of a normal, untreated rat kidney. Hematoxylin-eosin stain; × 100. *B*, section of irradiated rat kidney, showing an association of cortical scar and thyroid-like arrangement of hyaline-cast-filled tubules. PAS-orange G; × 100.

2. *Kidney in Irradiated Rat.*—The mammalian kidney was considered to be relatively radioresistant, although Hartman et al.[13] and Page[14] obtained arteriosclerosis and nephrosclerosis by roentgen irradiation in dogs, and isolated cases were reported in man (Dean and Abels,[15] Kunkler[16]). In spite of generalized arteriosclerosis and progressive nephrosclerosis mentioned by Casarett and his co-workers[17,18] in the rats treated with intravenous injection of polonium, Furth and his associates[19,20] found no changes in the kidneys of irradiated animals prior to 1954. In 1954, however, Furth et al.[21] reported some malignant nephrosclerosis late after irradiation. Other authors, including Billings et al.,[22] Berdjis and his co-workers,[10-12] Lamson et al.,[23,24] and others, reported a varying degree of nephrosclerosis in irradiated animals.

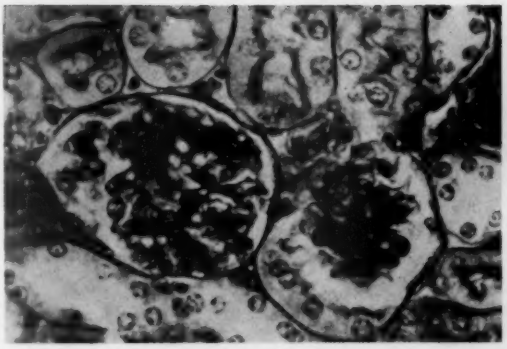

Fig. 2.—Photomicrograph showing two glomeruli in a cortisone-treated rat kidney, exhibiting intercapillary thickening. Note partial involvement of Bowman's capsule and relatively normal tubules at this stage. PAS-orange G; × 475.

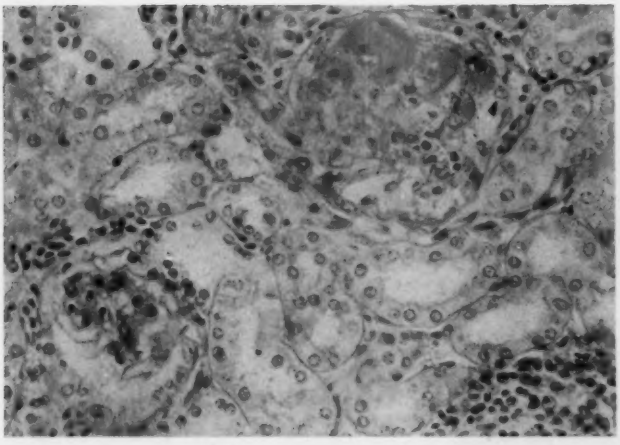

Fig. 3. — Glomerulosclerosis in a cortisone-treated rat, showing nodular hyaline masses in the glomeruli with partially patent capillaries. Hematoxylin-eosin stain; × 375.

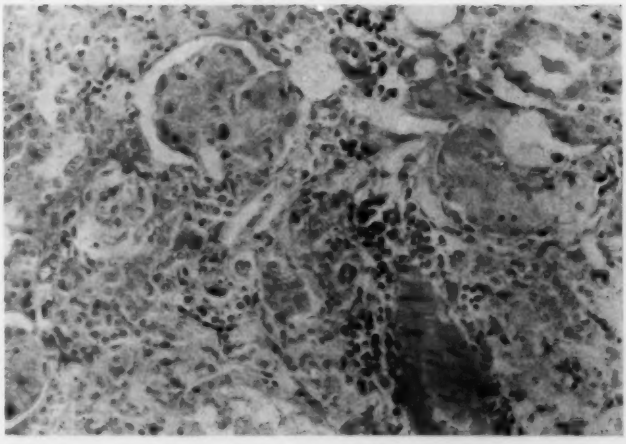

Fig. 4.—Photomicrographic section of a cortisone-treated rat kidney. The glomerular tuft is partly hyalinized, partly foamy, and partly vacuolated. Hematoxylin-eosin stain; × 300.

The changes occurring in kidney 100 days after exposure to 3×500 r of x-rays are summarized here: small or large cortical scars with concomitant tubular atrophy or dilatation (Fig. 1), partial or total fibrous atrophy of the glomeruli in these areas, and diffuse or foci of round-cell infiltration are outstanding figures. Widespread thickening of the basement membrane and glomerular capsule with focal dilatation of the tubules, and sometimes thyroid-like arrangement of the tubules containing hyaline casts, are frequent (Fig. 1).

3. *Kidney in Cortisone-Treated Rats.*—The glomeruli were the most affected by cortisone. The glomerular tufts exhibited thickening of the capillary walls or interstitial fibrosis (intercapillary thickening of certain authors). It also showed vacuolation and minute nodular formation. The changes occurring in the glomeruli could arbitrarily be divided in three stages. (*a*) Intercapillary thickening was the first stage (Fig. 2). (*b*) Nodular lesions, more or less hyalinized, with patent peripheral capillaries, represented the second stage (Fig. 3). (*c*) The third stage was some kind of exudative lesion, in which the glomerular tuft was partly hyalinized, partly foamy, and partly vacuolated (Fig. 4).

Whether or not these three types of glomerular change are stages in a continuing process could not be demonstrated with certainty. Nevertheless, in serial sections of

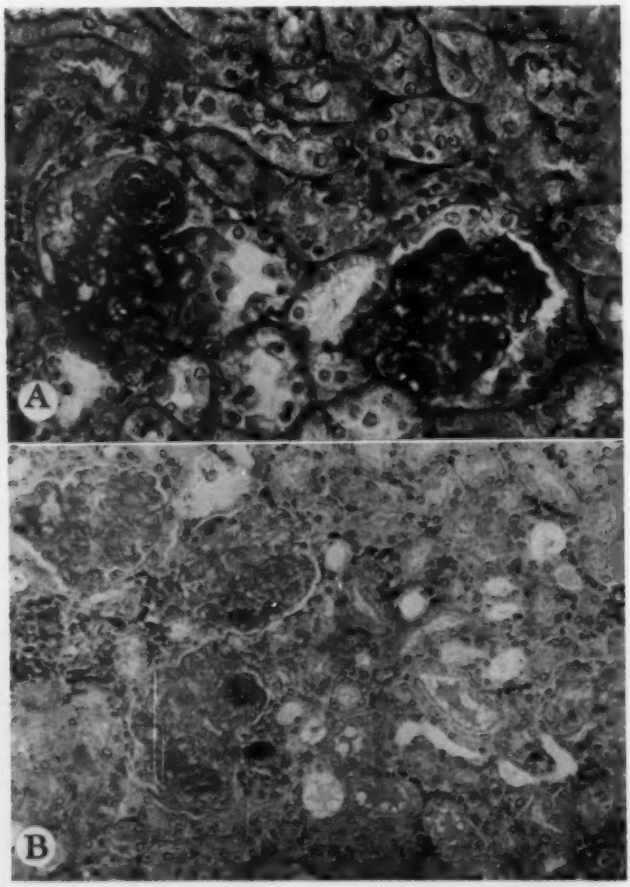

Fig. 5.—*A*, irradiated cortisone-treated rat kidney showing capillary thrombosis, thickening of capillary basement membrane, fusion of capillary loops, and production of hyaline PAS-positive masses; ×375. *B*, the same as in *A*. Hematoxylin-eosin stain; ×275.

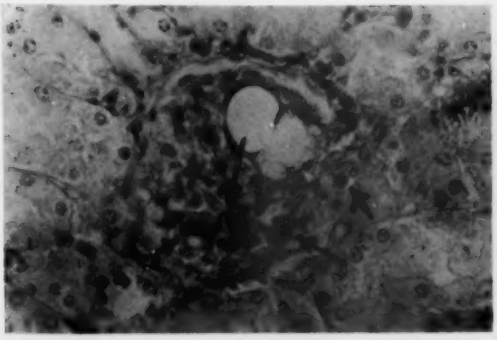

Fig. 6.—Irradiated cortisone-treated rat kidney exhibiting vacuolation with minute fat globules (arrows) and empty cystic formations in a glomerulus. Hematoxylin-scarlet red stain; × 375.

kidneys it was found that these lesions were present side by side in almost every case. More investigations with serial sacrifices at regular intervals are needed to confirm this process.

Although a certain degree of protein exudation was present, there was no evidence of cellular proliferation. This picture distinguished the present lesion from the other kinds of glomerulonephritis, including human chronic glomerulonephritis.

4. *Kidney in Cortisone-Treated Irradiated Rat.*—A. Glomerular Alteration: Capillary thrombosis or ischemia, thickening of capillary basement membrane, and production of fibrosis, leading to fusion of loops and/or capillaries, were the prominent pictures (Fig. 5). As the result of this impairment, crescent formation, hyaline masses, and diffuse or focal scars of glomerular tufts were conspicuous figures. Some of these scars or hyaline masses simulate "wire-loop" or crescent of some specific lesions. Obliteration of lumens of some capillaries, and possibly other mechanical and pathological factors, including increased permeability of the capillaries, led to dilatation of some capillaries, so that frequently the glomerular tufts were more or less vacuolated (Figs. 6, 7). Although some of these vacuoles contained minute fat globules (Fig. 6), others were cystic and devoid of any particles. Special stain for fat and glycogen and the routine stains failed to show any substance in some of these cystic formations. Periodic acid-Schiff stain, however, revealed some PAS-positive material (Fig. 5).

B. Tubules: Secondary tubular alteration was essentially due to blood-vessel impairment. Glomerular atrophy, thickened adherent capsule, and interstitial fibrosis led to atrophic renal parenchyma. Subsequently, some tubules were atrophic, others dilated. Groups of distal convoluted tubules contained protein or dense hyaline casts. Mod-

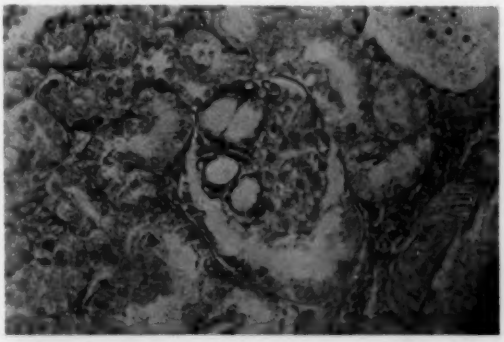

Fig. 7.—The same as in Figure 6. Hematoxylin-eosin stain; × 375.

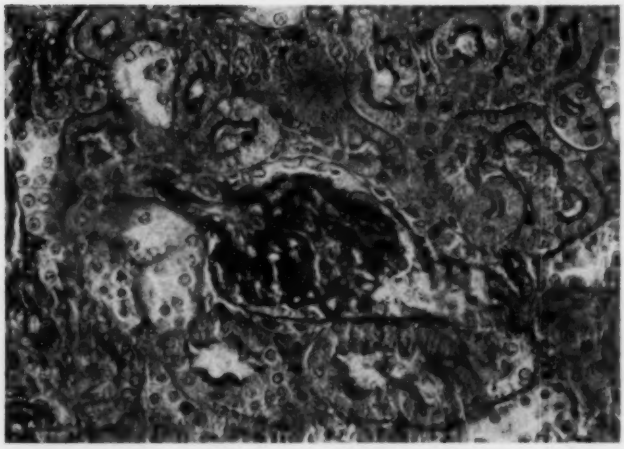

Fig. 8.—Irradiated cortisone-treated rat kidney, showing severe involvement of the entering (afferent) arteriole and hyaline masses with capillary fusion in a glomerulus. PAS-orange G; × 375.

erate to marked tubular changes illustrated secondary or primary tubular alteration, which reflected the degrees of glomerular damages (Figs. 4, 5, and 9).

C. Blood Vessels: Intense congestion and petechial hemorrhages were usual vascular disorders in the kidneys of cortisone-treated animals. While the glomerular capillaries exhibited thrombosis, with or without hyaline masses, occlusion, and cystic formation, the renal arterioles underwent varying degrees of arteriosclerotic changes (Fig. 8; also Figs. 3, 4).

Comment

Although the weight was not significantly affected, the size and shape of the kidney varied somewhat from animal to animal, as did the color and consistency, especially in the irradiated kidneys. These variations were primarily due to the frequent cystic formations in the kidneys of irradiated animals.[12,23]

While cortisone affected the glomeruli, especially the capillaries, the irradiation seemed to affect the renal parenchyma as a

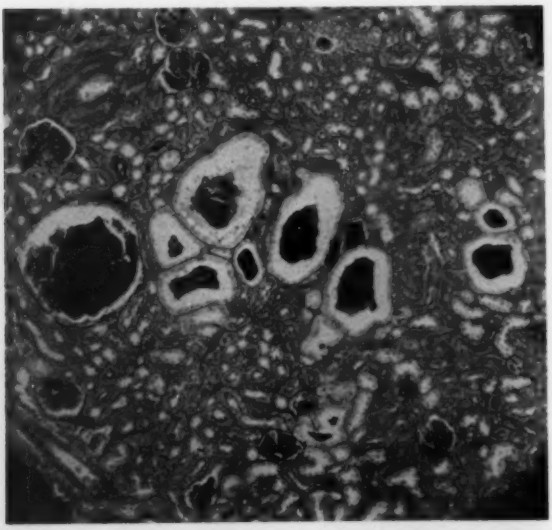

Fig. 9.—Photomicrograph illustrating marked tubular atrophy in a cortisone-treated irradiated rat kidney, with numerous dilated or cystic tubules containing colloid casts. PAS-orange G; × 100.

whole. For some reason that we cannot explain, the effects of radiation were not homogeneous, some kidneys being more affected than others, and some appearing to be refractory to the effect of x-irradiation. These observations are in close agreement with those of Lamson et al.[23,24]

It is of interest to note that the renal lesions, nephrosclerosis, glomerulosclerosis, or renal changes, reported here were applied to a generalized renal disease affecting both kidneys. In each kidney a great number of glomeruli were destroyed or impaired. The x-rays and cortisone seemed to be responsible for these changes, for no rat kidney in a control animal before two years shows such a general disease as described here. Our animals were roughly 6 months old, and the kidneys of such young animals were generally free of any glomerular or vascular diseases.

The most interesting and striking observation was that cortisone reinforced the injurious effect of irradiation in the kidney in affecting the glomerular tufts and the vascular system.

It appeared, therefore, that with respect to glomerular lesions in cortisone-treated animals, the results described here agreed with earlier reports,[3-7] but they did not agree with the findings of Wilens and Stumpf.[7] These authors and others stated that nodular and fatty glomerular lesions had been observed in the glomeruli of rabbits receiving cortisone. Wilens and Stumpf[7] further stated that cortisone administration had not been shown to produce similar lesions in any other species, including man. However, in spite of this statement, I did observe similar lesions in this present study on the rat glomeruli following cortisone administration. It is true that the fatty glomerular lesions were not an outstanding finding, for they were only occasionally present and were scattered, sometimes stippled or dusty-like, instead of globular fat or lipid masses as described by Wilens and Stumpf.[7] Nevertheless, the fat particles were present in some glomeruli of the cortisone-treated animals and in none of the irradiated animals.

Another curious effect of cortisone in kidneys was the presence of cystic formations in the glomerular tufts. Wilens and Stumpf[7] thought that they were empty spaces containing fat. Others, including Rich et al.,[6] interpreted them as microaneurysms. Although the fat globules were occasionally found in our animals, and the cysts were frequently present, it is doubtful whether this finding can be attributed to the empty fat lobules. With respect to the previous study on lungs[2] and the findings of Rich et al.,[6] it appeared that these cysts might be enlarged thrombotic capillaries in which the thrombi were absent. Furthermore, this material, when present, was strongly PAS-positive, as illustrated in Figure 5.

The glomerulonephritis, induced by cortisone and irradiation simultaneously, was composed of partial necrosis of glomerular tufts with fibrinoid alteration of capillary walls, concomitant intercapillary thickening, and partial secondary thrombosis of their lumens. As the result, there were hyaline type, foamy type, and vacuolated type of glomerular alterations or associated lesions leading to cystic formations (microaneurysms of certain authors[6]) and/or nodular hyaline masses. The last-mentioned lesions, associated with localized irregular scars, have been mistaken for "wire-loop" or crescent of some specific lesions. Certain authors, including Bloodworth and Hamwi,[4] described a similarity between glomerulosclerosis due to cortisone and diabetic glomerulosclerosis, Kimmelstiel-Wilson[25] type. Although a certain similarity existed, the "homogeneous, relatively acellular, hard collagen of the diabetic lesion with its sharp outline is not difficult to distinguish (Allen[26])." The hyaline thrombi and verrucal lesions of the capillary walls of the glomeruli may also be mistaken for wire loops. However, the hyaline masses or hyaline nodules of the cortisone-treated animals contained fat particles. They were also PAS-positive, while the so-called wire loops

did not respond to fat stain, and they were not homogeneously stained with PAS. Furthermore, as shown by Allen [27] and others, the argyrophilic pattern of each of these lesions is altogether different.

Summary

Two groups of young rats received 3×500 r of x-ray radiation in both kidneys. Half of these animals were given 3 mg. of cortisone daily for a period of 100 days. A third group received only cortisone, and a fourth group, untreated and nonirradiated, served as the control. While the control kidneys showed no alterations, the cortisone-treated animals developed glomerulosclerosis with intercapillary thickening, capillary thrombosis, hyaline masses, and cystic formations. These lesions were more marked in the irradiated groups.

Irradiation seemed to reinforce the injurious effect of cortisone in the kidney by affecting especially the glomerular and vascular system.

The action of cortisone upon the kidney appeared to be elective in the glomerular tufts and the blood vessels.

Arterio- and arteriolonephrosclerosis were outstanding findings in irradiated cortisone-treated rat kidney.

Fat particles were present in some glomeruli of the cortisone-treated animals, and in none of the irradiated animals.

Presence of fat and tinctorial affinities of the glomerular lesions and their interrelationship with some specific lesions (diabetes and lupus erythematosus) are discussed.

I wish to thank Mrs. Dorothy F. Shelton, Virology Division, Fourth U.S. Army Medical Laboratory, for her assistance in administering cortisone to the animals.

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The Mechanism of Cerebral Contusions

A Pathologic-Anatomic Study

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When brain lesions due to blunt forces are mentioned, they are usually referred to as contusions of the cerebral and cerebellar cortex resulting from either coup or contrecoup forces. The mechanism of coup lesions obviously never posed any particular problem. The contrecoup lesions, however, constituting a somewhat paradoxical phenomenon, have become the subject of many experimental and theoretical studies since, in 1766, the Académie Royale de Chirurgie in Paris offered a prize for *"établir la théorie des contrecoups dans les lésions de la tête et les conséquences qu'on peut en tirer."*

All efforts aimed at clarification of the mechanism of contrecoup contusions have led to some confusion, as evidenced by the existence of at least six different theories, so aptly illustrated by Pudenz and Shelden.[1] None of these theories has remained uncontested. One of the reasons for this is related to the pathology of brain injuries. It has attracted greater interest only in the last few decades. But even then one type of traumatic injury, the cortical contusions, has received most of the attention, while lesions in the deeper structures have gone almost unnoticed. When mentioned, they were not thoroughly analyzed and utilized for the problem at hand. Lesions which developed secondarily as the result of increased intracranial pressure or of a fall in systemic blood pressure, and sometimes as the sequela of fat or air embolism, have been, and still are, mistaken for primary traumatic changes.

It is, therefore, appropriate to examine once more how much evidence for the nature of the traumatizing mechanism can be collected by a more careful analysis of all types of primary lesions found at autopsy. The clarification of the mechanism leading to the so-called "contrecoup" lesions is only one of the objectives of this investigation. Just as important is the often-overlooked problem of why in most cases of a fall on the head coup lesions are absent or very minor in spite of severe contrecoup damage, whereas in cases of a blow to the movable head it is the contrecoup lesions which are rare and less extensive than the coup lesions. Furthermore, inquiry will be made into the probability of certain traumatic lesions being caused by a mechanism other than that responsible for "contrecoup" lesions. Gunshot cases showing contusions remote from the bullet tract will be referred to for clarifying the mechanism of certain observations.

There is an inherent disadvantage in attempting to draw conclusions from pathologic findings in the brains of victims of head injuries in regard to the physical changes responsible. The two essential variables, namely, the magnitude and the direction of the impact force, are rarely known. It is therefore necessary that the cases selected for a study like the present one fulfill certain requirements. They must be uncomplicated by nontraumatic conditions and only one impact must have been received; the type of impact, whether it was a blow or a fall, must be known; the site of impact must be established by clinical and/or anatomic findings; the type of instrument

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used in inflicting the blow or the nature of the object struck in a fall must be known; in regard to the injuries, skin lesions must be described and skull fractures recorded and illustrated in diagrams or photographs. In regard to the brain lesions, the investigator must separate the "primary" traumatic lesions, caused by the mechanical forces, from "secondary" lesions, due to post-traumatic circulatory disorders, especially those resulting from compression of vessels due to increased intracranial pressure (Lindenberg [2]). Among the primary lesions, the "contusions" resulting from mechanical stress within the tissues themselves must be differentiated from "wounds" caused by foreign objects, including bone fragments. Among the cortical contusions, one must separate the actual coup-contrecoup lesions from those in other locations, and possibly of other origin, e.g., lesions caused by skull fracture remote from the area of impact. In evaluating the primary traumatic lesions, it must be kept in mind that the morphologic phenomena of cerebral contusion, hemorrhages and necroses, occur at the moment of impact, and only then when the force transmitted to the brain exceeds a certain magnitude (Lindenberg and Freytag [3]); that their pattern of distribution is most significant for the present problem; that the size of a cortical contusion necrosis is determined by the magnitude of force acting on the cortex at the moment of impact and does not change during survival, but that a contusion hemorrhage often increases in size, depending on blood pressure and other factors effective during the time of survival. In drawing conclusions from the findings in regard to their probable mechanism, one must consider not only the various physical events known to be precipitated by the impact but also certain anatomic facts peculiar to the skull and its content which may facilitate or inhibit morphologic damage. Some of these factors follow. The eccentric support of the head by the spinal column; the spherical shape of the cranium, and the differences in the thickness and elasticity of its components; the irregularities of the floors of anterior and middle cranial fossae, as well as the smoothness of the interior surface of the cranial vault; the subdivision of the cranial cavity into three communicating compartments by falx and tentorium; the topographical relationship of cerebral vessels to firm structures, especially to the tentorium, and the fixation of the brain to the dura along the superior longitudinal sinus by the Pacchionian granulations of the arachnoid.

The principal physical changes against which the findings in the brain have to be checked are the following: First, acceleration of the head, which in blows to the resting, but movable, head is initiated by the impact, which in falls takes place prior to the impact, and which is absent when the head is firmly supported on the side opposite the area struck; second, changes of intracranial pressure related to the inertia of the brain during acceleration of the head; third, indentation or flattening of the skull at the site of impact; fourth, momentary displacement of brain tissues caused by this deformation of the skull; fifth, over-all deformation of the skull caused by the elastic local depression, and sixth, vibration of the skull and waves of compression within the brain originating at the site of impact and traveling with the speed of sound.

The cases used for this presentation were selected from more than 900 cases of fatal head injuries. They were grouped in three categories according to the status of the head at the moment of impact: First, cases in which a blow was inflicted to the movable, but not accelerated head; second, cases in which the head, accelerated in a fall, struck a firm object, and third, cases in which the head was supported and, therefore, not movable at the time it received the impact.

I. Cases with Impact to the Movable, Not Accelerated Head (Blows)

It is a common experience that in blows to the movable head the main brain damage is found at the site of impact and that contrecoup lesions are either missing or

less pronounced than the coup lesions. Courville [4] questions the occurrence of typical contrecoup lesions in man unless the head was in motion at the time of impact. However, in our material of 25 patients suffering solely from a blow to the head, 12 showed lesions in the areas of contrecoup, and 3 in other areas remote from the site of impact. Four of these cases will be described in greater detail, starting with one with the severest lesion.

CASE 1.—A 25-year-old laborer was working on an emery wheel, when suddenly the wheel broke. A piece of it struck the left side of his forehead, causing a depressed skull fracture above the left eye and an extensive laceration of the left frontal pole by penetrating bone fragments. At operation, these fragments and a part of the lacerated frontal lobe were removed. The patient died 24 hours after the injury.

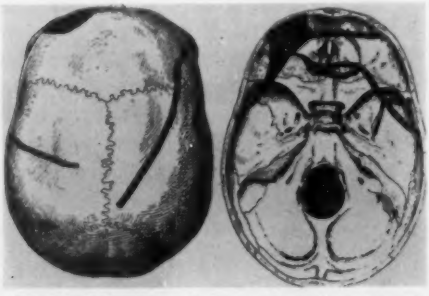


Fig. 1 (Case 1).—Diagram of skull fracture in calvaria and base.

Autopsy revealed a large defect in the left lower half of the frontal bone, and the anterior and middle cranial fossae exhibited fractures extending from the defect into the calvaria (Fig. 1). In the brain, there was an operative defect of the left frontal pole and of the lateral portion of the orbital region, whereas the right frontal lobe showed no significant injury. Consecutive coronal sections through the brain revealed an interesting distribution of the hemorrhages. In the left frontal lobe just posterior to the operative defect, the entire gray and white matter was interspersed with a multitude of hemorrhages of all sizes, and the orbital white matter showed a tissue defect filled with coagulated blood. The right frontal lobe was intact except for a few contusion hemorrhages in the cortex of the paramedial orbital region. At the level of the anterior commissure (Fig. 2), there were still innumerable hemorrhages in the gray and white matter of the left hemisphere

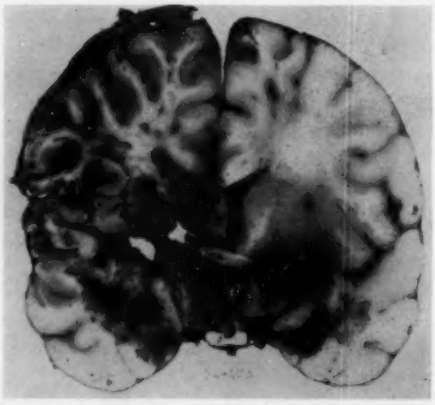
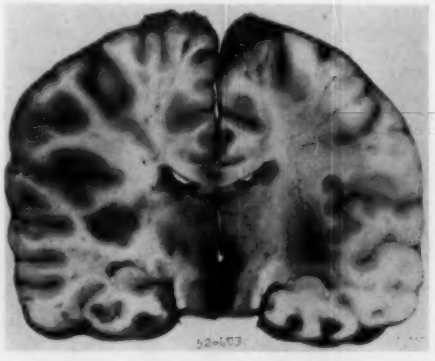


Fig. 2 (Case 1).—Section at level of optic chiasm, showing innumerable contusion hemorrhages in cortex and white matter of left hemisphere, spreading over midline to right anterior perforated substance, pallidum, and uncus. The second and third temporal convolutions at right were protected by lesser sphenoid wing and remained intact.

except for the second and third temporal convolutions, which were apparently protected by the lesser wing of the sphenoid. The corpus callosum showed hemorrhages limited to its left half. Between the anterior commissure and the optic chiasm, the hemorrhages spread over the midline, involving the anterior perforated substance, the pallidum, and the uncus of the right hemisphere. This hemisphere showed some additional contusions in the cortex of the first frontal and cingulate gyri. On a section through the mammillary bodies, the distribution pattern of the hemorrhages was seen to be essentially the same, but their number had decreased in the left centrum semiovale. Again, the lower temporal convolutions at the left were

Fig. 3 (Case 1).—Section of level of lateral geniculate bodies, showing hemorrhages fewer than on preceding section but still in same distribution.



spared, although the surrounding structures were most severely damaged. On a section at the level of the lateral geniculate bodies (Fig. 3), the posterior hypothalamus, the midportion of the midbrain, and the caudal part of the left putamen still showed marked hemorrhages; but the hemorrhages in other areas had decreased considerably in number, consisting mainly of cortical contusions in the left first temporal and central convolutions, in both hippocampal gyri, and in the paramedial portions of the central convolutions. A contusion in the right cingulate gyrus opposite the edge of the falx was associated with a hemorrhage in the center of the corpus callosum. In spite of the hemorrhages being reduced in number, their distribution pattern was practically the same as that on previous sections. Toward the posterior parietal and occipital lobes, this pattern changed (Fig. 4). Here, the majority of the contusions were located in the right hemisphere, involving predominantly the parieto-occipital and the lower lateral temporo-occipital cortex. At the left, the

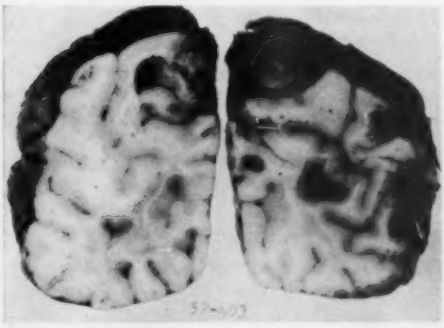


Fig. 4 (Case 1).—Section through occipital lobes, showing that most of the hemorrhages are in the right hemisphere.

cortical hemorrhages in the medial parietal and lower temporal areas decreased in number toward the occipital pole, which showed practically no damage. The lower midbrain showed a hemorrhagic necrosis in its paramedial portion. In the upper pons, there were symmetrical hemorrhages in the brachia conjunctiva and the tissues around the lateral sulci (Fig. 5), accompanied by multiple small hemorrhages in the subarachnoid spaces and the tissues proper of the rostral cerebellum. Finally, multiple small hemorrhages were present in the lower ventral portions of both cerebellar hemispheres facing the occipital bone without involving the cerebellar tonsils (Fig. 6).

A few of the many lesions were not caused by the impact and are immaterial for the analysis of the case. These were some of the hemorrhages in the rostral portion of the left frontal lobe resulting from surgery, the hemorrhagic necrosis in the midline of the lower midbrain, and some of the hemorrhages in the tegmentum of the upper pons, representing after-effects of vascular compression in the interpeduncular fossa. All other lesions were related to the impact force.

Fig. 5 (Case 1).—Symmetrical hemorrhages in branchia conjunctiva and tissues around lateral sulci of upper pons.

What conclusions can be drawn from localization and arrangement of the lesions in regard to their mechanism? It is self-explanatory that the left frontal lobe, carrying the brunt of the impact, was most severely damaged. But there was no less damage in the striate bodies and pallida,

Fig. 6 (Case 1).—Multiple small hemorrhages in lower ventral portion of both cerebellar hemispheres, facing occipital bone.

especially at the left, in both hippocampal gyri, and in the tissues around the third ventricle. Even the contralateral parieto-occipital lobes showed multiple contusions, but at this level they were more pronounced on the right than on the left. The continuity of all these lesions and their arrangement along a line extending from the left frontal region into the right occipital area indicate that the sudden caudal displacement of the left frontal lobe led to a sudden shift of the tissues predominantly along the line of impact direction toward the right occipital lobe, setting up shear strain of traumatizing degree within the brain and causing the surface of the brain to hit against tentorium, falx, and inner table of the occipital skull. Transmission of the shifting force through the tentorial aperture resulted in hemorrhages in the brachia conjunctiva of the upper pons, and the impact of the lower temporal lobes against the tentorial plane caused hemorrhages in the rostral cerebellum. The subsequent downward shift of the cerebellar hemispheres led to contusions in their lower ventral portions. On the other hand, no significant damage occurred in those convolutions of the left temporal lobe and of the convexity of most of the right cerebral hemisphere, because these areas were shielded by the left lesser wing of the sphenoid and by the falx, respectively.

The only lesions still to be accounted for are the multiple smaller contusion hemorrhages at the convexity of the rostral half of the cerebral hemispheres, extending from the left first temporal convolution to the middle third of the right convexity (Fig. 3). They are evidently related to the fracture of the calvaria, because the area involved by them corresponds very well to the skull segment bordered by the two longitudinal fracture lines. These lines must have developed when the rostral calvaria was bent outward, at the moment the frontal bone became depressed. Since they did not join, the piece of bone surrounded by them must have flipped back against the brain. As we concluded from findings in other cases of skull fracture, such elastic back-flipping produces cortical contusions only if the brain is in close contact with the bone, as it must have been in the present case.

Although the two mechanisms, impulse-like mass shifting of the brain and elastic back-flipping of the bone, satisfactorily explain all primary lesions in the present case, it should be checked whether any other mechanism could have influenced the development of at least some of the damage.

One may assume, for instance, that the blow to the forehead abruptly accelerated the head in fronto-occipital direction, whereby the frontal bones pushed the frontal lobes, while the occipital bone pulled away from the occipital lobes, causing a negative acceleration pressure over this contralateral area. In milder, nonpenetrating blows such intracranial-pressure changes related to the inertia of the brain significantly influence the extent of coup and contrecoup lesions, as will be demonstrated in subsequent cases. In the present one, the acceleration was very likely less pronounced because of the penetrating nature of the injury, but, nevertheless, may have somewhat enhanced the extent of the damage at the site of coup and very likely reduced the degree of contrecoup by the negative acceleration pressure, reducing the force with which otherwise the occipital lobes were slammed against the inner table of the skull. This negative acceleration pressure certainly did not produce the occipital lesions.

On the other hand, the fracture of the calvaria suggests that deformation of the skull took place in the form of an outward bending of the vault. Whether this led to a significant sudden shortening of the fronto-occipital axis of the skull, so that the occipital bone would have slapped the underlying brain, is doubtful, since any such fracture dampens or interrupts progression of over-all skull deformation. On the contrary, the negative pressure or vacuum produced by the outward bending of the bone must have produced a sucking effect on the underlying brain, distracting some of the shifting force, and in this way must have reduced the force of contrecoup.

The skull fracture also suggests that skull vibration can be excluded as a factor contributing to contusion. Any fracture stops or severely dampens propagation of vibration (Friede[5]). Finally, the perforating nature of the impact excludes compression waves traveling through the brain and linear or rotational oscillations of the brain within the skull as causative mechanisms of contusions, especially of contrecoup lesions.

Considering the multitude of lesions in this case, it is obvious that the usual terms "coup" and contrecoup" contusions must be supplemented by other terms characterizing lesions which are caused neither by coup nor by contrecoup but which are just as well primary traumatic lesions. The lesions in the left frontal lobe are the coup lesions. The hemorrhages at the convexity and lateral base of the right occipital lobe are the contralateral contrecoup lesions, and the contusions in both hippocampal gyri and in the cortex of both parieto-occipital and temporo-occipital areas may be called the same, since they resulted from the brain hitting against tentorium and posterior falx. The hemorrhages in the lower ventral cerebellum are actually also due to contrecoup but are not located contralaterally to the side of impact, since the shifting force was deflected by the tentorium. Therefore, they may be called "contrecoup lesions by deflection" or "heterolateral" contrecoup lesions. This type of contusions is not rare, especially not in cases with impact to the forehead; therefore, it is justifiable to characterize them by a special term. Furthermore, there were many hemorrhages in deeper structures of the brain which were neither coup nor contrecoup lesions. Since they were located between coup and contrecoup areas, we shall call them "intermediary coup lesions," the word "coup" signifying their direct relationship to the impact. Additional contusions indirectly caused by the impact are designated according to their immediate mechanism. Those caused by fractures of the skull remote from the impact area are called "fracture contusions." In the present case, this term would apply to the cortical hemorrhages beneath the fractures of the calvaria. Some other types of indirect contusions, to be described later, are "cerebellar herniation contusions," consisting of hemorrhages in cerebellar tonsils and adjacent medulla oblongata, caused by sudden wedging of the tonsils into the foramen magnum, and "gliding contusions," found in cortex and white matter adjacent to the superior longitudinal sinus and caused by the Pacchionian granulations exerting pull on veins in gliding movements of the brain.

Summary.—This case demonstrates, first, the necessity of differentiating not only between coup and contrecoup lesions but also between other types of contusions. Second, it proves that a mass shifting of the brain, most pronounced along the line of impact direction, is responsible for "intermediary coup lesions," due to shearing force in deeper structures of the brain, for "contralateral contrecoup contusions," due to the brain's hitting firm structures lying in the line of impact direction, and for "heterotopic contrecoup lesions," due to deflection of the shifting force by the tentorium. Third, it demonstrates that contusions may occur in tissues hitting smooth structures, like the plane of the tentorium, and that "fracture contusions" remote from the site of impact may complicate the anatomic picture. Fourth, the case suggests that the negative pressure over the contralateral surface of the brain resulting from the inertia of the brain to sudden acceleration of the head caused by the blow is the most essential factor in reducing the force with which the brain would hit the contralateral firm structures. Fifth, such reduced force of contrecoup still may produce cortical contusions but leave no gross changes in the nearby deeper white matter. Sixth, falx and tentorium—and in the present case the lesser sphenoid wing—may act as baffle but may also transmit some force to the brain tissues adjacent to their reverse side.

The next two patients suffered less extensive brain injury than the previous one. Both showed coup and contrecoup, but no

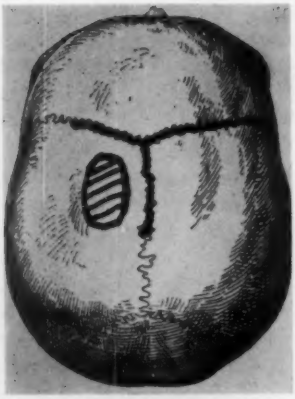


Fig. 7. (Case 2).—Diagram of skull fracture in calvaria.

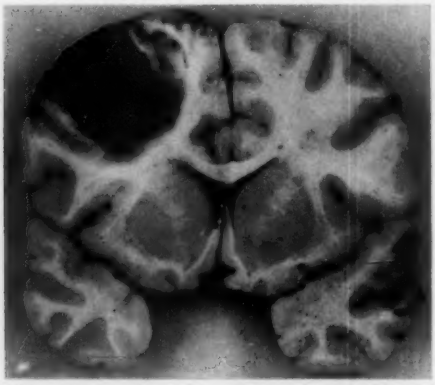


Fig. 8 (Case 2).—Coup lesion in left precentral area; contrecoup lesion in right temporal pole.

intermediary coup, lesions. In either instance, the impact was inflicted with an instrument, a baseball bat and a garden hoe, respectively. In blows of this type one must keep in mind that not all of the kinetic energy will affect the brain, because of rebound of the weapon and energy-consuming fractures of the skull, which occurred in both cases. There was no second impact to the head when the victims fell to the ground subsequent to the blows.

Case 2.—A 16-year-old youth received a blow with a baseball bat against the left parietal region, causing a circumscribed depressed fracture and a separation of the coronal and longitudinal sutures (Fig. 7). Exploratory trephines were made in front of the depressed fracture and over the right frontal lobe. The left anterior ventricular horn was tapped with a needle. The patient died 33 hours after the injury.

In contrast to the first case, no bone fragments penetrated the meninges. Underlying the impact area there was an oval, circumscribed subarachnoid hemorrhage covering the middle third of both central convolutions and the adjacent area of the second frontal convolution. In the right hemisphere, there were a few very small, dot-like subarachnoid hemorrhages, indicating a cortical contusion in the anterior third of the first and second temporal convolutions. On coronal sections, the coup lesion appeared as a large hemorrhage in the left hemisphere (Fig. 8), destroying the cortex and deeper white matter. It was most pronounced in the precentral portion of the second frontal convolution and became more superficial toward the parietal lobe. In the right hemisphere there was a medium-sized, almost triangular-shaped contusion necrosis, interspersed with hemorrhages, in the first and in the adjacent portion of the second temporal convolution, near the pole. Posteriorly 1 cm., the lesion terminated in the form of a few small intracortical hemorrhages. Still further posterior there was a second small cortical contusion hemorrhage, limited to the third temporal convolution. These contusions were obviously contrecoup lesions. The hemorrhage in the right second frontal convolution and the hemorrhage in the left cingulate gyrus were due to operation.

The physical events which must have taken place in this case within the few milliseconds after the baseball bat got in contact with the head were very likely the following: At first, there must have been an elastic depression of the skull in the area of contact immediately before the skull fractured. Its spreading over the skull must have been dampened by the fracture and the separation of the sutures. Therefore, any out- or inward bending of the skull at the side contralateral to the impact was absent, or of minor degree. Subsequently, the depression of the skull must have caused just as rapidly a displacement of the underlying brain, and therefore a momentary increase in intracranial pressure. Since the displacement must have been greatest underneath the point of maximal skull depression, it may be assumed that those portions of the deeper brain tissues lying in the direction of the impact were more displaced than were peripheral portions, and that therefore the impulse-like pressure increase was great-

est over the contralateral surface of the brain. At the time or shortly after the skull was fractured and the shifting of the brain was already taking place, acceleration of the head caused by the blow must have reached its peak. During acceleration, the skull must have pushed the brain over the side of impact and must have pulled away from it over the contralateral side. Therefore, the surface of the brain underlying the area of impact must have been exposed to two positive pressures, one caused by the indentation and the other by the acceleration of the skull. Contralaterally, the negative acceleration force must have counteracted the positive pressure force resulting from brain shifting. Assuming that over the contralateral side the two forces reached their maximum at about the same time, the one of greater magnitude must have exerted stress on the surface of the brain. Since experimental studies, to be discussed later, have shown that in impacts above a certain magnitude the positive pressure at the contralateral side is always greater than the negative acceleration pressure, it may be assumed that the positive shifting pressure—though reduced by the negative acceleration force—was still sufficiently high to cause the contrecoup lesions. The conclusions drawn in Case 1 support this assumption. It is also strengthened by the fact that in the present case the subarachnoid hemorrhage over the area of contrecoup was limited in extent, in spite of the fair size of the contrecoup necrosis. If the contrecoup lesion had resulted from the skull pulling away from the brain during the acceleration, more of the subarachnoid vessels would have been torn, causing a more widespread subarachnoid hemorrhage. Furthermore, the almost triangular shape of the contrecoup necrosis on cross section indicates that it resulted from positive pressure, as we discussed in a former paper (Lindenberg and Freytag [3]). As far as a rotational movement of the brain is concerned, it is very unlikely the cause of the contrecoup lesions, because the falx would have prohibited any major movement in the coronal plane, and lesions due to oscillations of the brain in the horizontal plane would have been located in other areas.

Summary.—The analysis of Case 2 suggests, first, that contrecoup contusions are produced by the brain slapping the skull as a result of an impulse-like shifting of the brain following the momentary depression of the skull by the blow; second, that the shifting is most pronounced within the line of the direction of the impact, and that, therefore, the site of the contrecoup lesions is mainly determined by the impact direction; third, that two positive forces, namely, impact and positive acceleration forces, act at the site of the blow, and that the positive shifting force arriving at the contralateral side is reduced by a negative acceleration force. This would explain why in cases of a blow to the head the contrecoup lesions are usually less extensive than the coup lesions, and are often missing.

CASE 3.—A 35-year-old man got into an argument, was struck with the back of a garden hoe over the left lateral forehead, and "sank" unconscious to the blacktop pavement of a parking lot. He was not operated on except for a few stitches over his forehead and survived about 15 hours.

At autopsy, the only external injury seen was a longitudinal laceration and bruise of the skin extending almost parallel to the midline from the midportion of the eyebrow over the forehead into the hair line. The injury was interrupted midway by a bridge of intact tissue. This pattern is explained by the shape of the hoe: The laceration above the eyebrow was caused by the shaft; the one in the hair line, by the hook of the hoe. The shape of the unsutured bruise suggests that the blow was directed perpendicularly to a tangent drawn through the area struck. Underlying the skin injury, there was a fresh hematoma over the skull, extending toward the left temporal area. No other subcutaneous hemorrhages were found. The skull revealed a longitudinal tension fracture running halfway between the impact area and the midline, parallel to the latter and extending from the lambdoid suture through the frontal bone into the left and right orbital roofs, without bone displacement (Fig. 9). There was little subdural hemorrhage in either middle cranial fossa. The convexity of the left frontal lobe and medial portion of both orbital lobes were covered with fresh subarachnoid hemorrhage. On coronal sections, multiple traumatic lesions were observed in

the rostral half of both cerebral hemispheres (Figs. 10, 11). Subjacent to the site of impact by the shaft of the hoe, there were multiple small hemorrhages in the cortex of the lateral convexity of the left rostral frontal lobe. Underlying the impact area of the hook, there were two triangular-shaped contusion necroses in the caudal third of the left second frontal convolution (Fig. 11). In the lateral portion of the left frontal pole, the coup lesions were continuous with contrecoup lesions involving both rostral orbital regions (Fig. 10). Exclusive of some postmortem laceration in this area, the left orbital lobe appeared to be more damaged than the right, especially in its lateral part. Further caudally, the contrecoup contusions were limited to both gyri recti and their vicinity. A small wedge-shaped contusion necrosis in the precentral third of the right third frontal convolution was apparently a contrecoup contusion related to the impact by the hook (Fig. 11). The laceration in the cortex of the left temporal lobe was

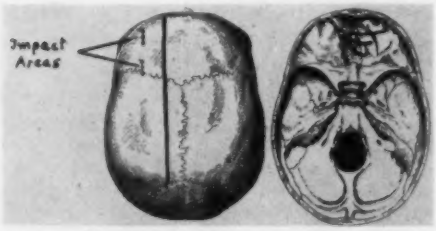


Fig. 9 (Case 3).—Diagram of skull fractures in calvaria and base.

an autopsy artifact. The caudal half of the brain revealed no lesions.

This case is somewhat unique in that the one blow resulted simultaneously in two separate impacts, one by the hook and the other by the shaft of the hoe. Both produced not only their own coup lesions but also their own contrecoup lesions. The contrecoup destruction of the orbital convolutions, which often results from a fall on the back of the head, can be explained in the present case only by an abrupt shift of the frontal lobes from the site of impact by the shaft toward the orbital roofs. Near the left lateral frontal pole, the coup lesions blended with the contrecoup lesions. The latter were unusually extensive because the short distance between coup and contrecoup areas did not allow for any absorption of the shifting force by brain tissues. Furthermore, the irregularities in the surface of the

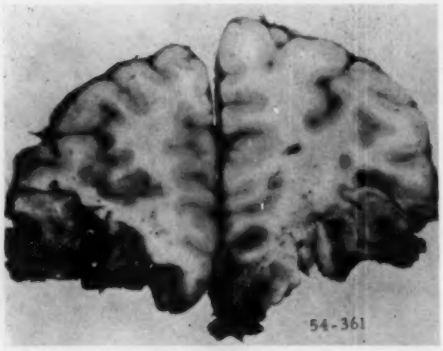
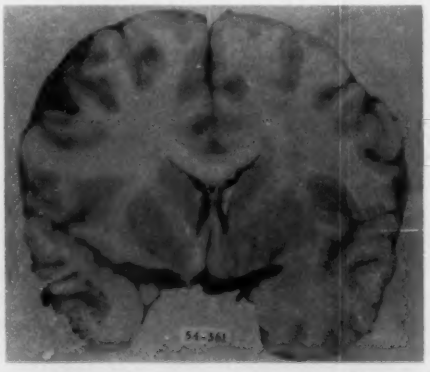


Fig. 10 (Case 3).—Section through frontal lobes, showing contrecoup lesions in both rostral orbital regions, due to impact by the shaft of the hoe.

orbital roofs, the paucity of cushioning cerebrospinal fluid in this area, and the fracture facilitated the contrecoup damage. On the other hand, the distance between the areas of coup and contrecoup related to the impact by the hook is greater, and, therefore, the brain could absorb some of the shifting force caused by this impact. If a line is drawn between the latter coup and contrecoup areas, it roughly corresponds to the direction of impact as it would be postulated on the basis of skin injury. It is, therefore, assumed that the major shifting of the brain occurred along this line, and that by passing through the opening of the

Fig. 11 (Case 3).—Section at level of striate bodies, showing two triangular-shaped coup lesions in left second frontal convolution, due to the impact by the hook of the hoe. Small wedge-shaped contrecoup lesion in precentral third of right third frontal convolution.



falx, it reached the contralateral surface of the brain. Its force must have been reduced by the aforementioned absorption and by some negative acceleration pressure, but was still strong enough to produce the contrecoup necrosis. Because of its location in the lower frontal instead of in the temporal lobe, because of the limited subarachnoid hemorrhage accompanying it, and because of the fracture of the skull, no other mechanism would convincingly explain the contrecoup damage. In regard to the skull fracture, it may be pointed out that it must have been due to an outward bending of the skull, exceeding the limit of elasticity of the bone. In contrast to Case 1, the elastic return of the bone to normal position caused no fracture contusions. The vacuum created by the outward bending of the skull very likely absorbed some of the shifting force by distracting it from the line of impact direction.

Summary.—Case 3 supports the conclusions drawn in the two former cases in regard to the coup-contrecoup mechanism. In addition, it demonstrates the significance of the length of distance between coup and contrecoup areas as a factor influencing the intensity of contrecoup force: The shorter the distance the less shifting force will be absorbed.

CASE 4.—This case is of special interest because it takes an intermediary position between cases with coup-contrecoup lesions and those with only coup lesions. There were lesions remote from the site of impact, but these lesions were not actual contrecoup lesions. They consisted of hemorrhages in the cerebellar tonsils and medulla oblongata, suggesting that a gliding of the brain toward the foramen magnum took place.

A 24-year-old man was struck over the right frontoparietal region with an iron bar during an argument. He died instantly, without hitting his head when falling to the ground.

Autopsy revealed a deep laceration of the scalp above the right ear. The underlying skull showed a depressed fracture measuring 1½×2 in. Bone fragments had pierced the dura. There were small amounts of subdural and subarachnoid hemorrhage in the area of the impact. A coronal section of the brain through the center of the impact area (Fig. 12) showed some depression of the right anterior central region, a localized subarachnoid hemorrhage, and multiple contusion hemorrhages in the cortex subjacent to the area struck. There was no trace of contrecoup effect in the cerebral hemispheres. However, both cerebellar tonsils and the dorsal portion of the medulla oblongata revealed innumerable fresh hemorrhages in the brain substance, obviously responsible for the sudden death (Fig. 13).

What mechanism could have caused the hemorrages in this particular location, and so remote from the site of impact, without producing contrecoup lesions in the cortex facing the orbital roofs or the tentorium? One could assume that it was a vibration of the skull which, being amplified at the point of the support of the head by the spinal column, produced the hemorrhages in the tissues adjacent to the foramen magnum. But this theory would not explain why the

Fig. 12 (Case 4).—Section through center of impact area, showing depression of right anterior central region and multiple contusions in its cortex. No contrecoup lesions.

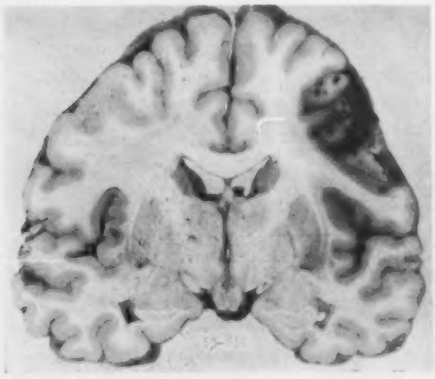
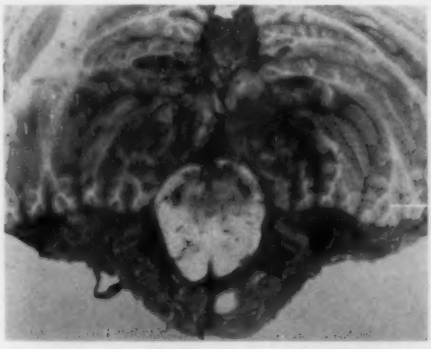


Fig. 13 (Case 4).—Herniation contusion with multiple hemorrhages in both cerebellar tonsils and dorsal portion of medulla oblongata.



hemorrhages extended into the deeper portions of the tonsils and why they were located in the tegmentum of the medulla oblongata, and not in its periphery. Furthermore, the vibration could not have been of significant intensity, since the fracture resulting from the blow was a depressed one and dampened any propagation of vibration. Perhaps, a sudden shortening of the vertical axis of the skull occurred, and the medulla oblongata, together with the cerebellar tonsils, was squeezed into the foramen magnum, while the other portions of the brain experienced no significant movement. This theory would explain the absence of any cerebral and cerebellar contrecoup lesions in the vicinity of the tentorium, as well as the distribution pattern of the hemorrhages in tonsils and medulla. However, considering the depressed skull fracture, it is questionable whether any essential shortening of the vertical axis of the skull took place. Any such shortening would have resulted in an elongation of its horizontal perimeter, a deformation which the brain would have followed, thereby preventing the cerebellar tonsils from being squeezed into the foramen magnum.

It is of interest that we observed identical hemorrhages in the cerebellar tonsils in cases in which any shortening of the vertical axis of the skull could not have occurred. These cases were gunshot cases, and one of them will be briefly described. While lying on a couch, the patient shot himself with a .22 caliber gun. The bullet went through the palate and the orbital roof, and between the frontal lobes, and came to a halt over the frontal convexity, near the superior longitudinal sinus. Other than the small wound canal and a few hemorrhages in its vicinity, the only traumatic lesions were multiple hemorrhages in both cerebellar tonsils. These hemorrhages could not have been caused by vibration or by a shortening of the vertical axis of the skull, but must have been related to a sudden gliding of the brain radially away from the path of the missile. This gliding movement of the brain must have been imparted to the structures within the posterior cranial fossa, causing the cerebellar hemispheres to glide toward the foramen magnum, the area of least resistance, and thus the cerebellar tonsils, to become momentarily herniated into the foramen magnum.

We believe that the same herniation mechanism was responsible for the hemorrhages in the present case (Fig. 13). Since this mechanism differs from those hitherto discussed, we call these lesions "herniation contusions." In contrast to contrecoup contusions, which occur in the cerebellar tissues facing the occipital bone (Case 1), herniation contusions are only indirectly related to the impact and are indicative not of the direction of impact but of a gliding of the brain toward the emergency exit of the skull. Such movement of the entire brain is possible only if it is not firmly pressed against the inner table of the skull at the moment of impact. With greater impact force the pressure will become so great that friction between brain surface and skull will not permit any gliding. This is, in our opinion, the reason that we seldom found a combination of contrecoup and herniation contusions in an individual case.

Summary.—Case 4 demonstrates, first, that increased intracranial pressure caused by the impact forces the brain to glide toward the foramen magnum as long as the pressure is not so great that it presses the brain against the skull and friction prohibits such gliding; second, that because of this mechanism, the medulla oblongata is the site of strain regardless of site and direction of impact, and, third, that no contrecoup lesions develop as long as the brain is able to perform such gliding

II. Cases with Impact to the Accelerated Head (Falls)

The injuries belonging to the cases of this category are usually received in a fall or when the victim is thrown against a firm object. The basic difference between these cases and those of the first category is that

the head is already accelerated when receiving the impact, whereas in the first group the head is stationary and then accelerated by the blow, and that the force of impact is usually greater than in blows. Otherwise, all physical events to be taken into consideration are essentially the same as those considered in the former group.

The phenomenon of contrecoup is usually demonstrated by a patient who fell on the back of the head and suffered no, or only mild, coup lesions but sustained severe contrecoup damage in frontal, orbital, and temporal lobes. Such a "classical" case, however, is not very conclusive in regard to the mechanism of contrecoup lesions because, as a rule, intermediary coup lesions are absent in this variety of fall. We, therefore, selected as our first case one in which the impact against the occipital region left distinct traces within the brain tissues. The distribution pattern of the brain lesions is similar to that seen in Case 1. In fact, this case must be regarded as a transitional one between the usual cases with blows to and those with falls on the head because it showed coup and intermediary coup lesions, but very few contrecoup lesions.

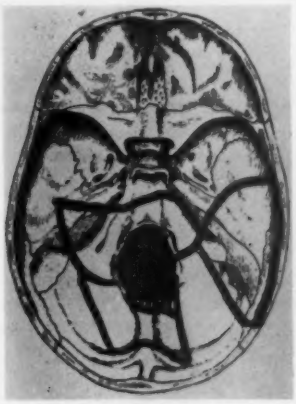


Fig. 14 (Case 5).—Diagram of skull fractures in base.

CASE 5.—A 28-year-old man jumped out of a moving train, received multiple fractures of ribs and extremities, and struck with the lower part of the back of his head a bolt of the neighboring track. He died immediately.

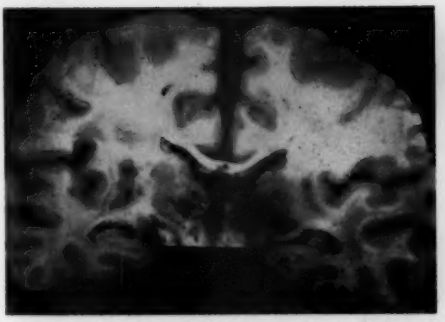
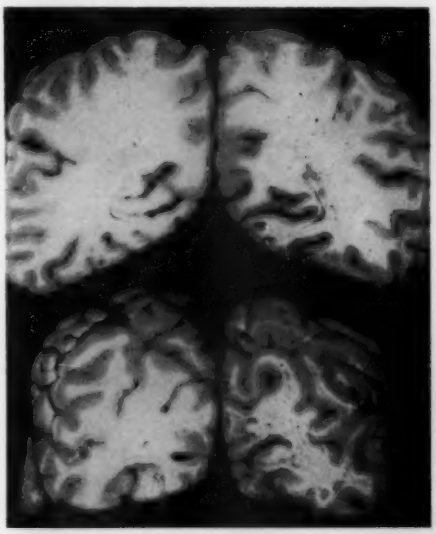


Fig. 15 (Case 5).—Section at level of lateral geniculate bodies, showing multiple hemorrhages almost exclusively located in right hemisphere and involving thalamus and temporal and occipital cortex facing the tentorium.

The skin showed a laceration in the right lower occipital region near the midline. The base of the skull was extensively fractured, and a small segment of the right dorsal rim of the foramen magnum was separated and depressed (Fig. 14). Opposite to this piece of bone, the cerebellum was superficially lacerated. The essential lesions within the brain were as follows: Near the impact area, there were multiple hemorrhages in both cerebellar tonsils, the lower vermis, and the lower paramedial convolutions. On a section through the

Fig. 16 (Case 5).—Sections through parieto-occipital region, showing contusions in lower temporo-occipital cortex and at convexity and medical aspect of right occipital lobe.



dentate nuclei, both these structures, the white matter, the cerebellar cortex, and the subarachnoid spaces between the folia showed multiple hemorrhages, more so on the right than on the left. Similar hemorrhages were found in the rostral part of the cerebellum and in the tegmentum of the upper pons. In the cerebrum, there were also multiple contusion hemorrhages, which were, however, almost exclusively located in the right hemisphere and involved the thalamus and the temporal and occipital cortex facing the tentorium (Fig. 15). In the right occipital lobe, additional contusion hemorrhages were found at its convexity and medial aspect (Fig. 16). In the entire rostral half of the brain no traumatic lesions could be found.

From the type of fall and the various fractures of ribs and extremities, it may be assumed that most of the impact force was taken up by the body, reducing its effect on the head. The head must have been bent forward in order to receive the impact near the foramen magnum. Furthermore, the impact area was small and circumscribed because the bolt measured not more than about 1 in. in diameter.

Regarding the brain lesions, the interesting feature of this case is that the innumerable hemorrhages were distributed within a cone of tissue having its apex at the site of impact and its base in the area of the right half of the tentorium, and that the convolutions of frontal poles, orbital lobes, temporal poles, and of the convexity of the cerebral hemispheres were intact except for those of the right occipital lobe.

The simplest explanation of this pattern of distribution would be that the inbending, fracturing, and depression of the bone at the site of impact caused an impulse-like shift of the cerebellum mainly in the direction of impact, which therefore must have been predominantly toward the right half of the tentorium. The rostal cerebellum must have transmitted the impulse to the tentorium, and the tentorium, in turn, to the adjacent temporal convolutions. In the region of the tentorial opening, the traumatizing effect of the shifting extended above the level of the tentorium into the thalami, especially into the right one. Further dorsally, the force must have been so much reduced by tentorium and brain tissues that contrecoup contusions were limited to the convexity of the right occipital lobe, being closest to the tentorium. The absence of contrecoup lesions over all other portions of the convexity may be attributed to the smoothness of the inner table of the calvaria, permitting the brain to glide without becoming contused.

An attempt to explain the occipital contrecoup lesions, and perhaps also the contusions in the lower temporal convolutions, by a "falling through" of the brain is futile in view of the fact that the intermediary contusions in the upper brain stem extended above the tentorium into the thalamus. The distribution pattern of the lesions offers, furthermore, no indication that rotational or linear oscillating movements of the brain within the skull could have caused or contributed to the lesions. The severity of the skull fracture speaks against a significant shortening of the vertical axis of the skull or skull vibration having taken place.

Summary.—Case 5 permits the conclusions, first, that in a fall sudden depression of the skull at the site of impact causes an abrupt shift of the brain, which is most pronounced in a cone-like mass of tissue, the apex of the cone pointing to the site of impact and its axis corresponding to the direction of the impact; second, that contusions of cortex facing the smooth surface of tentorium and cranial vault occur only if the shifting force is sufficiently great to prevent gliding; third, that rotational movements of the brain, elastic shortening of the vertical axis of the skull, and skull vibration do neither cause nor contribute to the lesions.

The next case provides further evidence that an impulse-like shifting of the brain takes place in the direction of impact and demonstrates that this shifting is responsible for contrecoup contusions.

Case 6.—An 18-year-old youth was a passenger in an automobile, sitting beside the driver. The driver presumedly fell asleep, and the automobile, after crossing the street, hit a telephone pole. The passenger was thrown against firm structures of the car in front of him and suffered an impact against the rostral vertex. He survived this injury in an unconscious condition for about two and a half days.

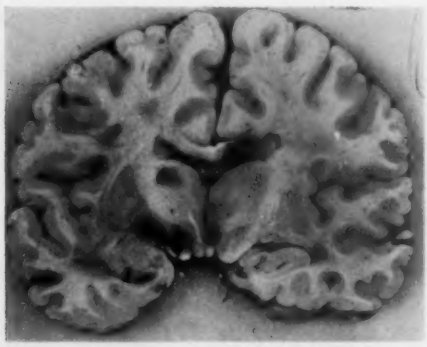


Fig. 17 (Case 6).—Section at level of mammillary bodies, showing contusions in both hippocampal gyri opposite the edge of the tentorium, in vicinity of mammillary bodies, and in anterior half of corpus callosum.

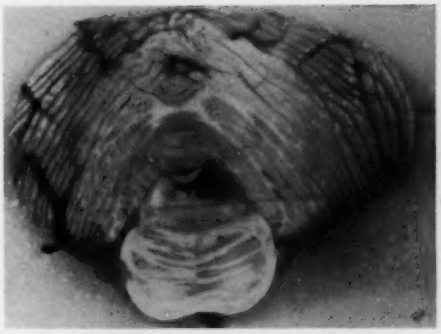


Fig. 18 (Case 6).—Contusion hemorrhages in left brachium conjunctivum of upper pons.

Autopsy revealed a fresh hematoma in the galea over the rostral vertex, indicating the site of impact. The underlying skull showed no fracture, but several fracture lines were found in both orbital plates, without displacement of fragments, and a transverse fracture extended through both petrous bones and clivus without radiating into the calvaria. There was little subdural and subarachnoid hemorrhage over the central and upper parietal areas of the right cerebral hemisphere. On coronal sections, multiple small and fresh contusion hemorrhages were seen irregularly distributed throughout the cortex of both orbital lobes, in both hippocampal gyri opposite the edge of the tentorium, and bilaterally in the caudal temporal cortex facing the plane of the tentorium (Fig. 17). Further traumatic lesions were found in the right half of the anterior corpus callosum, in the vicinity of the mammillary bodies, and in the left brachium conjunctivum (Fig. 18). A small softening in the dorsal portion of the left thalamus was secondary in nature, and not essential for the following analysis.

Before we discuss the findings in this case, we must briefly consider what happens to a passenger of an automobile which, traveling at a uniform rate of speed, is suddenly decelerated by running with its front into an object. Prior to the collision, gravitational but no acceleration force acts on the person. At the moment of collision, his body tends to move forward with the speed of the vehicle. Thus, one could assume that no acceleration is involved when the person strikes a firm object in front of him. In reality, the forward motion of the person is not free and undisturbed because the gravitational force, i.e., the weight of the body, causes friction wherever the body is in contact with parts of the car, especially between the body and the seat. In other words, the decelerating car pulls the seat from under the body. Since the spinal column is flexible, this sudden, and eccentric backward pull results in a violent forward acceleration of the upper part of the body, especially of the head, a split second prior to collision of the head with the windshield or any other firm structure. It is this acceleration to which we refer in the discussion of the present as well as the next case.

In contrast to the foregoing case, this one showed neither a fracture at the site of impact nor coup lesions in the brain. However, there were fractures in the orbital roofs and a transverse fracture in the petrous bones and clivus, indicating that the skull was appreciably deformed at the moment of the impact. The absence of coup lesions may be attributed to the protective cushion of cerebrospinal fluid, which is usually thicker at the convexity than at the base of the brain. Furthermore, the acceleration of the head prior to the impact caused negative acceleration pressure beneath the impact area and possibly increased the thickness of this cushion and absorbed a further portion of the impact force. On the other hand, at the contralateral side positive acceleration force very likely brought the basal portions of the

brain closer to the orbital roofs and the tentorium and enhanced the shifting force of the brain, thus facilitating the development of the contrecoup hemorrhages in orbital and lower temporal lobes. A few of these lesions may have been fracture contusions. The arrangement of the hemorrhages in the vicinity of the mammillary bodies and their association with uncal contusions can have resulted only from a sudden wedging of the tissues into the tentorial opening, while the difference is that coup lesions are more freing by the tentorium. In analogy with the cerebellar herniation contusions previously described, they may be called "tentorial herniation contusions," but since they are indicative of impact direction in the present case, they are actually intermediary coup lesions. This tentorial herniation is also responsible for the lesion in the left brachium conjunctivum, which, in our opinion, is a primary traumatic one, due to a sudden caudal shifting of the upper pons relative to the rather stationary cerebellum, tearing intrapontine twigs of a branch of the superior cerebellar artery which supplies this specific region of the upper pons. The mechanism of the corpus-callosum lesion must have been not only the ventral displacement of the brain but also a simultaneous stretching of the corpus, due to a short-lasting, elastic elongation of the transverse axis of the skull at the moment of impact. Since the lateral portions of the cerebral hemispheres must have followed this deformation, it must have slightly reduced the contrecoup effect of the shifting force by distracting some of it laterally from the line of impact direction. This, and the baffling effect of the tentorium, very likely prevented contusion of the lower surface of the cerebellum.

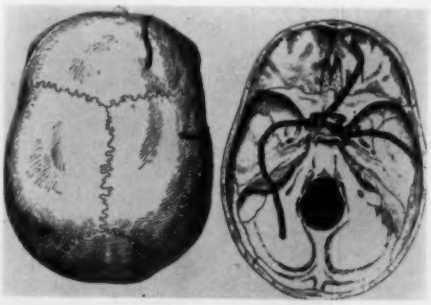
Summary.—This case demonstrates, first, that a shifting of the brain in the direction of impact is the essential mechanism of contrecoup and intermediary coup lesions; second, that for lesions of the corpus callosum a simultaneous stretching, due to an elastic elongation of the transverse axis of the skull, is a contributory factor; third, that a shifting of the brain toward the tentorial opening causes primary damage in the lower hypothalamus, in its vicinity and in brachium conjunctivum by sudden temporary herniation; fourth, that negative acceleration pressure underneath the area of impact helps prevent coup lesions, whereas positive acceleration pressure over the contralateral side facilitates contrecoup contusions; fifth, that elastic deformation of the skull with elongation of its transverse diameter reduces the force of brain shifting and prevents contrecoup damage in lower cerebellum.

The following case constitutes a further step toward those cases with no or only small coup lesions and marked contrecoup lesions but no intermediary contusions. The type of impact was similar to that in the previous case.

CASE 7.—The 20-year-old man was a passenger sitting beside the driver. He was thrown forward when the automobile struck an electric pole and injured his right lateral forehead and face. He died on the way to the hospital.

At autopsy, the skull showed fracture lines in the right frontal and temporal bones, joining at the sella and crossing into the left half of the cranium, as indicated in Figure 19. There was no subdural and little subarachnoid hemorrhage over either hemisphere, being more pronounced over the left occipitoparietal region. The main findings were as follows: The caudal portion of the right orbital lobe, the adjacent temporal pole, and the left gyrus rectus showed multiple fresh contusion hemorrhages (Fig. 20). At the level of the tuber cinereum, there were a few small contusion hemorrhages in both unci and in the wall of the third ventricle. In the caudal half of the left hemisphere

Fig. 19 (Case 7).—Diagram of skull fracture in calvaria and base.



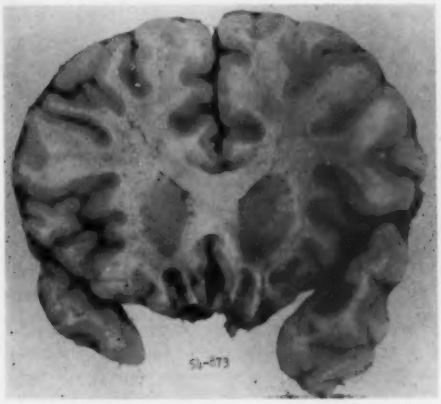


Fig. 20 (Case 7).—Section at level of striate bodies, showing multiple contusions in caudal portion of right orbital lobe, adjacent temporal pole, and left gyrus rectus.

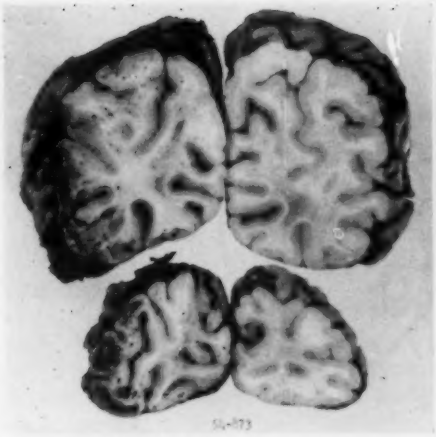


Fig. 21 (Case 7).—Sections through posterior parietal and occipital lobes, showing multiple contrecoup contusions in parietal and occipital cortex of left hemisphere.

(Fig. 21), multiple contusion hemorrhages involved the cortex of the parietal region, the caudal portions of the first, second, and third temporal convolutions, the convexity of the occipital lobe, and the lower aspect of the temporo-occipital region. The caudal half of the right hemisphere showed no contusions. There were no lesions in the infratentorial brain areas.

Impact area and direction in this case are similar to those in Case 1. However, in the present case the impact occurred to the accelerated head, involved the right instead of the left frontal region, and was obviously of less force per surface unit, because the skull fracture was not a depressed one. Furthermore, the lesions in the rostral half of the brain were rather limited in comparison with those in Case 1. There was not even a single coup lesion. Contralateral contrecoup lesions involved the parieto-occipital region of the other hemisphere, as they did in Case 1, but covered a much larger area.

The shape of the skull fracture suggests that the major impact hit the lateral portion of the right forehead, and that the frontal bone, right orbital roof, and lesser sphenoid wing, as one piece of bone, were temporarily wedged between the orbital lobe and the temporal pole, causing contusion hemorrhages in the orbital and temporal cortex around the right lesser sphenoid wing. Therefore, these cortical lesions are coup lesions and may more specifically be called "remote" coup lesions. The displacement of the bone must have resulted in an almost horizontal impulse-like shifting of the brain in a caudal direction, causing both unci to strike against the tentorial edge, thus undergoing contrecoup hemorrhages. The main displacement of the brain must have occurred along an axis extending from the right lateral frontal region to the left occipital region, because the extensive contrecoup contusions involved the caudal half of only the left cerebral hemisphere. The hemorrhages in the wall of the third ventricle were the result of shearing force, due to a shifting of the basal ganglia into the opening of the tentorium. The somewhat paradoxical finding of marked contrecoup lesions in the absence of coup lesions could be explained by taking into account the presence of negative and positive acceleration pressures over the areas of coup and contrecoup, respectively, at the moment of impact. A sudden elastic shortening of the diagonal axis of the skull, immediately preceding its fracturing, may have contributed to the contrecoup damage.

One could, of course, also theorize that, at the moment when the right forehead struck the firm object, the brain continued its accel-

eration in the direction of the right frontal bone and hit against the lesser sphenoid wing and the orbital roof while trying to pull away from the skull over the left temporo-occipital area. This mechanism would explain the contusions in the orbitotemporal cortex by positive pressure, and the contusions in the caudal portions of the left cerebral hemisphere by negative pressure or cavitation, but cannot account for the contusions in the unci and the wall of third ventricle, because the brain would have shifted away from the tentorial edge, leaving these structures intact. If one considers all findings, rotational movements of the brain or skull vibration likewise cannot have been of any significance in producing the contusions.

Summary.—This case demonstrates, first, that extensive contralateral contrecoup contusions may occur in the absence of coup lesions in the area underlying the impact; second, that irregularities in the inner table of the skull are no prerequisite for the development of contrecoup lesions; third, that location and extent of the contusions again suggest as mechanism an impulse-like shift of the brain away from the area of impact with maximal shift taking place along the line of impact direction; fourth, that the distribution of negative and positive acceleration forces is essential for the absence of coup and the presence of contrecoup contusions, and, fifth, that in impacts to the forehead, lesions in the vicinity of the lesser sphenoid wing may be "remote" coup lesions, simulating contrecoup lesions resulting from a fall on the back of the head. This last variant of injury will be discussed next.

As has been previously mentioned, the classical case, with impact to the back of the head, shows no or only limited coup lesions, but extensive contrecoup contusions in the orbitofrontal and rostral temporal regions without any appreciable coup lesions. But not in all of these cases are the contrecoup contusions distributed in this way. The variability in their distribution pattern may be illustrated by six cases. In Case 8 both orbital lobes were severely contused (Fig. 22). In Case 9 the main damage was in the left orbital lobe, whereas the right one showed only one typically triangular-shaped contusion necrosis in the medial portions of the gyrus rectus (Fig. 23). In Case 10 the contrecoup lesions were located in the convolutions of the ipsilateral frontal pole and adjacent rostral portions of the frontal convexity, completely sparing the orbital convolutions (Fig. 24). In Case 11 the contusions were predominantly distributed over the lower lateral aspect of the frontal convexity of the contralateral hemisphere (Fig. 25). In Case 12 all the lesions were confined to the cerebellum (Fig. 26). Case 5, already described, may serve as the last example demonstrating the fact that the rostral half of the cerebrum need not be involved.

Although in all six cases the same general area, namely, the occipital region, received

Fig. 22 (Case 8).—Contrecoup lesions in both orbital areas in a fall on the occiput.

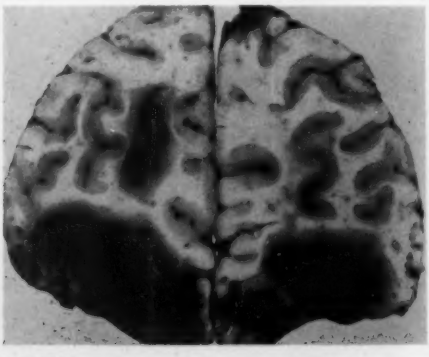
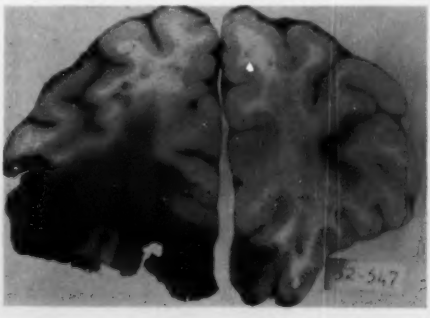


Fig. 23 (Case 9).—Contrecoup lesions in orbital areas, mainly of left side, in a fall on the occiput.



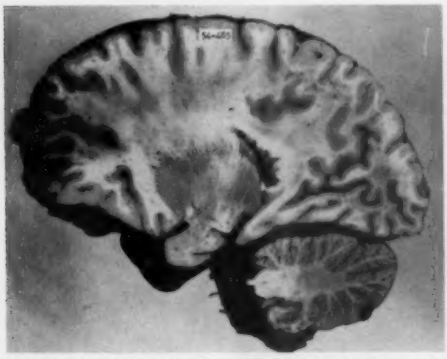


Fig. 24 (Case 10).—Longitudinal section through left hemisphere, showing large contrecoup lesions in frontal poles and small coup lesion in left lower occipital region in a fall on the occiput. Orbital lobes intact.

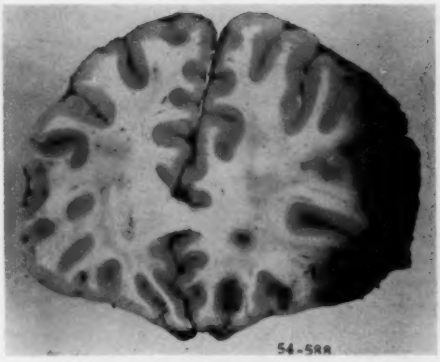
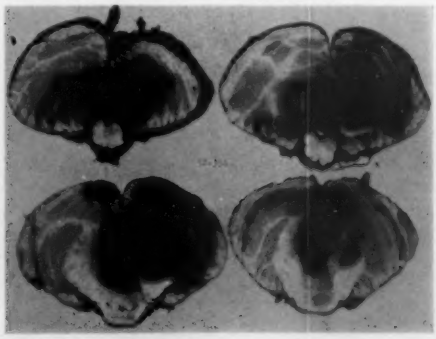


Fig. 25 (Case 11).—Contrecoup lesions at lateral aspect of right frontal convexity in a fall on the occiput.

the impact, the site of each individual impact varied from one case to the other, as shown diagrammatically in Figure 27. The relationships of these impact areas to the main areas of contralateral lesions are given by arrows in the two diagrams (Figs. 28 and 29), representing lateral and horizontal views, respectively. The great diversity in the directions in which the arrows point can only be explained by the direction of impact having varied from one case to the other. Therefore, the main factor in determining the site of contralateral lesions in an individual case must be the impact direction, as was concluded from the previous cases.

The mechanism responsible for the contrecoup lesions in Figures 22, 23, 24, 25, and 26 was, in our opinion, the same as that of the contrecoup lesions in Case 7. Had the lesions been caused by a falling through of the brain, some of these cases should have shown contusions in the unci, as a result of the brain falling against the tentorial edge. It is a common observation, however, that uncal contusions are very rare in all those cases in which the fall on the back of the head produced fronto-orbital contrecoup lesions and that they occur oftenest in cases with impacts to the vertex or forehead. This fact renders further support to the theory that the contrecoup lesions are due to the brain slapping against the skull, as described previously. The absence of any significant coup lesion in these cases is for the same reason as that given in the analyses of Cases 6 and 7. But, besides the protecting effect of

Fig. 26 (Case 12).—Contrecoup lesions in cerebellum in a fall on the occiput.



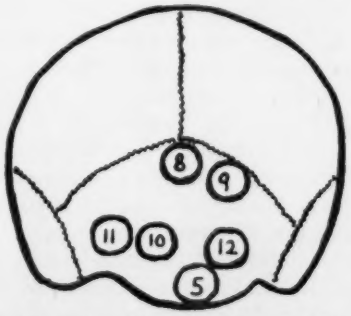


Fig. 27.—Schematic drawing of occipital area of skull, showing variability of impact areas in six cases of falls on the occiput. The numbers designate case numbers; the circles represent impact areas.

the negative acceleration pressure over the site of impact, there is probably an additional physical process which helps prevent coup lesions. From the moment the falling head gets in contact with a firm flat object, there will be a progressing flattening of the skull, starting at the point of initial contact and spreading centrifugally until the impact force is absorbed. This flattening must lead peripherally to a transient outward bending of the skull. Since this outward bending has some sucking effect on the underlying brain surface, it will absorb some of the positive pressure with which the brain presses against

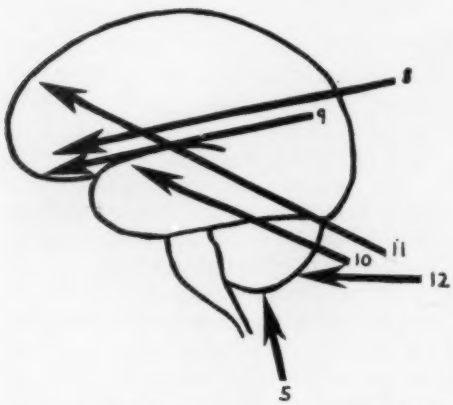


Fig. 28.—Schematic drawing of lateral view of skull, showing variability of impact direction in six cases of falls on the occiput. The numbers designate case numbers. The arrows connecting areas of impact with respective areas of contrecoup represent impact directions.

the inner table and will in this way help prevent coup lesions without adversely influencing the shifting of the brain along the line of impact direction. This shifting will proceed until the flattening of the skull reaches its maximum, and it is only then that the brain has the first chance to fall through. Although the process of flattening takes only about 1 msec., it is still part of the falling process. If the skull strikes an irregular object, it will be indented instead of flattened, and a coup lesion is more likely to occur. If the skull is fractured, the inner table breaks first, so that the coup lesions may be partially a fracture contusion.

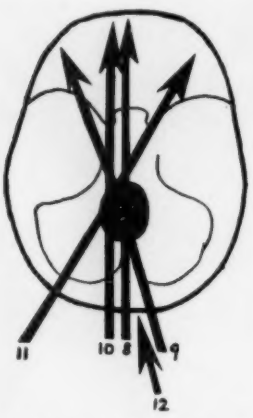
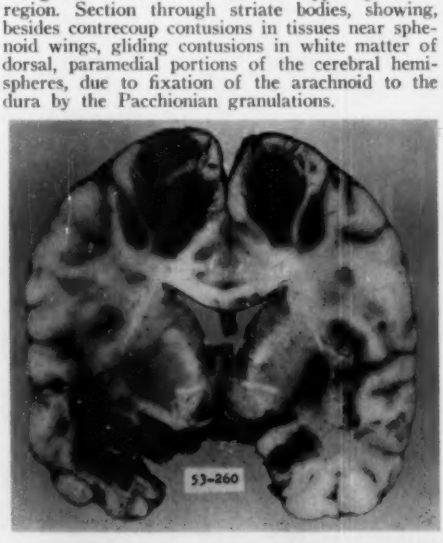


Fig. 29.—Schematic drawing of base of skull, showing variability of impact direction in six cases of falls on the occiput. The numbers designate case numbers. The arrows connecting areas of impact with respective areas of contrecoup represent impact directions.

In impacts to the temporal regions, essentially the same mechanisms take place as in falls on the back of the head. The only difference is that coup lesions are more frequent and are relatively larger than in impacts to other areas of the skull, not counting fracture contusions. This fact is probably due to a greater elasticity of the skull in the

Fig. 30.—Case of a fall on upper posterior region. Section through striate bodies, showing, besides contrecoup contusions in tissues near sphenoid wings, gliding contusions in white matter of dorsal, paramedial portions of the cerebral hemispheres, due to fixation of the arachnoid to the dura by the Pacchionian granulations.



temporal region, the thinness of the cushion of cerebrospinal fluid over the temporal lobe, and the relative fixation of the lobe within the middle fossa. Like the contralateral lesions, they rarely involve the dorsal convolutions of the convexity because of the protection offered by the falx, which in some cases is evidenced by contusion hemorrhages in the cingulate gyri, facing the margin of the falx.

One type of contusions occasionally seen in falls on the head deserves special attention. This is hemorrhages in the white matter of the dorsal paramedial portions of the cerebral hemispheres, as illustrated in Figure 30. These hemorrhages most frequently involve the white matter of both first frontal convolutions near the central region. The hemorrhages are predominantly venous in nature and are usually associated with hemorrhages and vascular necrosis in the paramedial cortex. Their location in the vicinity of the Pacchionian granulations suggests that their mechanism is somehow related to the fact that the arachnoid is fastened to the dura by these granulations. Therefore, the dorsal veins may be exposed to undue pulling stress if the brain performs gliding movements. In such case, the lesions may be called "gliding" contusions. If they are caused by coup or contrecoup forces, one usually finds wedge-shaped contusion necroses in the cortex facing the superior longitudinal sinus which are absent in typical "gliding contusions."

III. Cases with Impacts to the Supported Head

Bona fide cases of this category appear to be extremely rare. Cases in which the supported head is run over by a vehicle or in which the head was squeezed between two firm objects do actually not belong to this group because the mechanical force applied to the head does not have the nature of an impact, as in the cases of the first two categories. If the force of such pressure is sufficiently great to cause a fracture of the skull, bone fragments may produce lacerations, but contusion foci do not occur, as in one of our cases in which the head was pinned by a moving freight car against a standing one. In another case, a bulldozer rolled off its carrier and over the forehead of a man pinned to the ground. In spite of most severe fracturing of the anterior half of the skull, the brain showed neither contusions nor lacerations. In a third case, a 2½-year-old child was run over by a backing trailer truck and suffered severe fractures of the skull, especially of the anterior cranial fossa, with separation of the cribriform plate. At autopsy, the brain showed only lacerations of the orbital convolutions caused by bone fragments.

There are, however, cases in which the head received blows—sometimes more than one—with a firm object while being supported. Two cases may be mentioned as examples. In the first one, heavy blows were repeatedly inflicted to the forehead of the victim with an iron pipe while the head was resting against the back rest of an automobile. Autopsy revealed that the anterior half of the skull was severely fractured, but no coup or contrecoup lesions were present. There were only a few, and very small, fracture contusions in the cortex near the broken lesser sphenoid wing. In the second case, the head of the victim, while he was lying in bed, was struck repeatedly with a firm object. In spite of most severe fracturing of the floor of the anterior cranial fossa and fractures through the left middle and right posterior cranial fossae, there was only one small contusion hemorrhage in the right temporal pole, facing the fractured lesser sphenoid wing. Also in this case, coup and contrecoup lesions were absent.

The absence of coup lesions in both cases is very likely due to the following factors: First, a large amount of the force transmitted to the skull is absorbed not only by the fracturing of the skull but also by the supporting medium; second, in the resting head, there is a thick cushion of cerebrospinal fluid under the area of impact, because the brain, heavier than spinal fluid, is resting on

the contralateral inner surface of the skull; third, because of the support of the head, there is no appreciable acceleration, i.e., no positive acceleration pressure over the brain underlying the site of impact. The absence of contrecoup lesions may be explained as follows: First, at the moment when the skull is indented at the site of impact, there will be some indentation also at the site of support, depending on the consistency of the supporting medium; second, this bilateral deformation will simultaneously produce some elongation of the skull axis perpendicular to the line connecting the two points of indentation; third, the brain will follow this deformation and, by doing so, reduce any shifting of the brain in the line of impact direction. Only if the impact force exceeds a certain magnitude and the consistency of the supporting medium is rather low may some coup or contrecoup contusions be expected. We have no case which would support this assumption.

Comment

The occurrence and location of cerebral contusions, and their absence in other regions of the brain, is dependent not only upon the magnitude and direction of impact and the structural features and physical reactions of the skull but also upon the state of the head while receiving the impact, i.e., whether the head was stationary but movable (blow), accelerated (fall) or contralaterally supported. Each of these factors is variable and their combination differs from one case to the other; therefore, usually several different physical processes, synergistic or antagonistic in their action upon one another, are responsible for enhancing or inhibiting injurious forces and for determining the site of brain damage. This is the basic conclusion drawn from the cases presented.

In discussing details, we shall start with those cases in which the head was stationary, but movable at the time of impact, because many of the experimental data on the physical events taking place in skull and brain were obtained in applying blows to the heads of animals or of human cadavers.

One of these events was first experimentally demonstrated by Braquehaye[6] (1895) and later on by other investigators (Friede,[5] Walker et al.,[7] Gurdjian and Lissner[8]). The experiments proved that in blows to the movable head an initially positive pressure exists over the brain underlying the impact area and an initially negative pressure over the contralateral brain surface. The subsequent rapidly dampened pressure oscillation lasts about 0.02 second (Walker et al.,[7] Gurdjian and Lissner[8]). It is generally agreed that the negative pressure is due to the inertia of the brain being greater than that of the skull in sudden acceleration. At the side of impact the skull pushes the brain, and at the contralateral side it pulls away from it. Recently, Gross[9] demonstrated in model experiments that at the contralateral side not only such negative pressure but cavitations in the liquid content of a glass flask occur if the flask is suddenly accelerated by a sharp blow to the rubber stopper in its neck. Pudenz and Shelden[1] applied blows to the heads of monkeys in which the calvaria was replaced by a translucent plastic skull and, in this way, visually demonstrated that the acceleration of the head results in rotational gliding movements of the brain within the cranial cavity, the planes of rotation being dependent on the site and direction of impact. Such movements had been previously postulated and studied on gelatin models of the brain by Holbourn.[10]

As valid as these observations are, and as important they may be for the problem of concussion, are they also significant for elucidating the mechanism of brain contusion, especially of contrecoup lesions? Russell,[11] Dott et al.,[12] Friede,[5] and others believe that the initial negative pressure over the contralateral surface of the brain is responsible for the development of contrecoup lesions. According to Gross,[9] it is not the negative acceleration pressure which produces contrecoup hemorrhages but the imparting pressures resulting from the collapse

of minute cavities. Similarly, coup hemorrhages are supposedly due to the same mechanism initiated by the snapback of the bone indented by the blow. Holbourn [10] and Pudenz and Shelden [1] concluded that a shear strain in those areas of the brain surface, where irregularities of the skull prevent it from gliding, is responsible for contrecoup contusions.

In regard to the latter rotational theory, Courville [4] has already pointed out its inadequacy for explaining the mechanism of contrecoup injuries. In our opinion, it is also erroneous to relate these lesions to the initial negative acceleration pressure or to the collapse of cavities. In all of these experiments, the force of the blows was inadequate for causing morphologic contrecoup damage. Pudenz and Shelden,[1] for example, used only "subconcussive" blows. Besides, some of the experiments were designed for studying the mechanism of concussion and not of contrecoup (Walker et al.,[7] Gurdjian and Lissner [8]). Nevertheless, the intracranial pressure changes demonstrated by their experiments may lead to morphologic alterations other than contrecoup contusions. A tear in one of the bridging veins may cause subdural bleeding, as was shown by Pudenz and Shelden.[1] This may be especially so in elderly people with reduced strength of neck muscles, increased rigidity of skull, and some atrophy of the brain. In such cases, the impact may be so mild that it appears to be insignificant and, upon hospitalization of the patient, is hardly mentioned, or is even omitted in the preadmission history. This is one of the reasons that some investigators (Link [13] and others) believe that the greater number of old subdural hematomas are not traumatic in origin. We cannot agree with them because in our autopsy material trauma was the cause of nearly all subdural hemorrhages and hematomas, and in several cases the initiating impact appeared to have been trivial. In some of our cases the fall was on the buttocks and not on the head, but death was from large subdural hematomas. Sudden movement of the brain may also lead to subarachnoid hemorrhage of venous, and occasionally of arterial, origin. Thus, a fist blow may prove fatal by sudden, arterial subarachnoid bleeding without having produced cortical contusions. This is more likely to be so if the arterial walls are congenitally hypoplastic or if a vascular malformation, for instance, a berry aneurysm, is present. We agree with Courville [4] that the only intracerebral lesions which may result from brain displacement relative to indentation of cortex and white matter near the dorsal margin of the cerebral hemispheres, lesions which we call "gliding contusions" (Fig. 30).

It is now of interest that an increase in the force of the blow does not lead to a proportionate rise in the contralateral negative pressure. On the contrary, the pressure may be positive right from the beginning, as was shown by Gurdjian and co-workers,[14] who found in their experiments that this positive pressure could amount up to almost seven times that of normal atmospheric pressure and could last from 0.3 to 6 msec. The authors attribute this phenomenon to brain displacement relative to indentation of the skull. This observation is, in our opinion, the most important experimental contribution to the problem of the mechanism of contrecoup lesions. It fully supports the conclusion we drew from our autopsy findings that, basically, contrecoup lesions must be the result of the brain hitting firmer structures of the cranial cavity. However, we also concluded that the impulse-like pressure produces no contrecoup contusions if it does not exceed the limits of the elasticity of vessel walls and cellular membranes. Instead, the over-all pressure will force the brain toward and into the only emergency exit of the skull, the foramen magnum, causing shear strain in medulla oblongata and cerebellar tonsils, regardless of site and direction of impact. As morphologic evidence of such strain we found hemorrhages in cerebellar tonsils and dorsal medulla oblongata, which we called "tonsillar herniation contusions" (Fig. 13). They occurred not only with blows to the head but also in cases with small-caliber gunshot wounds of the cerebral hemispheres and were seldom accompanied by contre-

coup contusions but frequently by skull fractures and coup lesions. As the mildest form of such herniation, we found small, insignificant-looking hemorrhages in the subarachnoid space between and within the folia of the tonsils, while the medulla oblongata appeared to be grossly intact. The hemorrhages, however, strongly suggest that this vital structure must have been also under stress and that death may have been caused by fatal concussion if it cannot be explained by other findings. The microscopic finding of small extravasations in the medulla oblongata, especially in its dorsal half, and in the tissues of the tonsils, particularly if located in those facing the margin of the foramen magnum, is definite evidence in favor of such opinion. It seems to us unlikely that injurious herniation can result solely from acceleration or deceleration accompanying impacts, since the positive and negative pressure forces, produced by either one, act simultaneously and are balanced. They do not force the brain to leave the cranial cavity. For this, an over-all positive pressure appears to be a condition *sine qua non*.

Also, in experimental concussion, the tissues within and near the foramen magnum may have been found to be the site of predilection for hemorrhages (Jakob,[15] Denny-Brown and Russell,[16] Friede [5]). On the other hand, the experiments proved that such lesions need not be present in severe, and even fatal, concussion (Polis,[17] Denny-Brown and Russell [16]). In the search for a histologic substrate of concussion in such cases, it was found that during the postconcussion period neurons of various substantia griseae in the brain stem, especially of the reticular substance, often develop chromatolytic changes (Windle et al.,[18] Chason et al.[19]) which Friede [20] recently identified as "axonal reaction" to damage of their axons. He could demonstrate that in sudden changes of the position of the head the odontoid process is instrumental in producing the damaging strain in the cord, especially involving thick myelinated fibers, and that a subluxation of the process will enhance the damage. This mechanism is completely different from the herniation mechanism. The question arises whether it may occur in man. We are not aware of any suitable case which might have been described in the literature. In our collection, we have several cases in which hemorrhages exclusively involve the lower medulla oblongata and the upper cervical cord; however, since in every case the spinal canal was distorted by fracture and dislocation of the upper cervical vertebrae, the hemorrhages were attributed to compression. In all cases, the fractures occurred as the result of overbending of the head in falls. It is very probable, however, that the lesions described by Friede [20] will be found without fracture of the neck, once the pathologists are cognizant of their mechanism and pay more attention to the upper cervical segments, especially in cases which survived a whiplash injury for some days.

We may now discuss the mechanism of contrecoup lesions in blows to the head. The deeper the skull is depressed by the blow and the larger the area of impact, the greater will be the intracranial pressure resulting from displacement of the underlying brain tissue. At the moment of maximal skull indentation, this displacement will be most pronounced beneath the center of skull depression and of slighter degree toward its periphery. Because of the facts that the brain is not liquid, but semisolid, and that the displacement takes place in less than a millisecond, one may assume that the difference in the degree of displacement is not immediately equalized. Consequently, the impulse-like pressure with which the displaced brain is squeezed against the inner table of the skull will be initially greatest contralateral to the side of impact. As we concluded from the cases presented, it is this local pressure peak which is essentially responsible for contrecoup hemorrhages and necroses. Since the site of maximal depression is determined by the direction of the impact, the most pronounced displacement of brain will take place along the line of this

direction, or, as Courville[4] puts it, along the line of force. Therefore, the general location of contrecoup lesions is directly related to the direction of impact. Since a blow to the movable head causes also acceleration of the head, any positive pressure arriving at the contralateral surface of the brain will be diminished by the negative acceleration pressure which almost simultaneously develops. On the other hand, at the side of impact, the positive acceleration pressure will enhance the force of the blow. This synergistic relationship of the positive acceleration pressure to the force of impact and the antagonistic relationship of the negative acceleration pressure to the force of brain displacement or contrecoup are, in our opinion, the main reason that in blows to the movable head coup lesions are commonly more pronounced than contrecoup lesions, if the latter occur at all. Some authors underestimated, or even neglected, the skull depression as a factor essential for the development of contrecoup damage, but others put emphasis on it (Kocher[21] (1901), Doepfner,[22] Kalbfleisch,[23] Spatz,[24] Welte,[25] and others), and many of them believe that the skull deformation sets up more or less linear compression waves traveling through the brain to the contralateral side and causing contrecoup lesions by being reflected from the skull or dural membranes. In a former paper, we (Lindenberg and Freytag[3]), too, used the term compression waves, but we now believe that a sudden mass shifting of the brain, even if limited to the range of a millimeter, is by far more important than a compression wave, which need not be accompanied by a mass movement of the brain tissues.

Turning now to the mechanism of contusions in impacts to the already accelerated head, as is the case in falls, we do not know of any animal experiments which were performed to elucidate the physical changes taking place in this specific version of injury. Besides the fact that such experiments would obviously run into some recording difficulties, they seem to be unnecessary, since many authors believe that impacts to the head produce basically identical physical changes, regardless of whether they are afflicted by a blow or received in a fall. Indeed, experiments by Friede,[5] in which he dropped heads of human cadavers on a firm object, seemingly support such an opinion. The changes of intracranial pressure were the same as those he observed in blows to the movable head. There were initial positive pressure over the brain at the side of impact and a negative one over the contralateral side, followed by a short-lasting, rapidly dampened pressure oscillation. The initial pressures indicate that the brain continues to fall, though only for a fraction of a second, when the skull is suddenly decelerated, i.e., arrested in its forward motion. These initial pressures and their changes may occasionally cause those lesions which have been mentioned as possible sequelae of mild blows to the head, e.g., subdural and subarachnoid hemorrhages; but could they also be responsible for contrecoup contusions? According to Friede,[5] Russell,[11] Schneider,[26] and others, the answer is "yes." Just as these authors consider the initial negative acceleration pressure in blows to the head as cause of these lesions, they attribute them in falls to the initial negative deceleration pressure. We cannot agree with this theory. Friede's[5] experiments were no proof for it, because the heights from which he dropped the heads of cadavers did not exceed 25 cm., and, therefore, the impacts were of a rather mild nature. Thus, his experiments demonstrate only that in mild falls the pattern of intracranial pressure changes is identical with that observed in mild blows, although produced by deceleration, and not by acceleration, of the skull. If the negative acceleration pressure does not produce contrecoup contusions in blows, why should the negative deceleration pressure be able to cause them in falls? Furthermore, the first contusions which may result from a blow are not contrecoup but coup lesions; and only if the impact force exceeds a certain magnitude may contrecoup lesions occur. We pointed out that the initial contralateral negative acceleration pressure recorded in

mild blows does not become greater with increasing force of the impact; instead, it gives way to an initial positive pressure throughout because of the greater indentation of the skull, simultaneously causing a sudden shifting of the brain predominantly in the direction of impact. Only if this occurs, contrecoup lesion may develop. Essentially the same thing must take place in falls once the impact is so severe that the flattening of the skull is sufficiently great. Consequently, one should expect that also in falls first coup, and with greater impact force contrecoup, contusions would develop. But this is not the case. Contrecoup lesions dominate the scene, and coup lesions are often absent. Therefore, an additional and very essential factor must play a role at the moment of impact. In our opinion, it is the acceleration of the head taking place during the fall. While the person is falling, negative acceleration pressure will exist over the brain region next to the skull area going to receive the impact, and positive acceleration pressure over the contralateral brain surface. Because of the relatively long duration of acceleration in falls, the cerebrospinal fluid has time to shift according to the pressure gradient, so that at the moment of impact its cushion is thicker than normal at the side of impact and thinner than normal at the contralateral side. For the cerebral cortex, this provides better protection against the force of the coup by dispersing it over the entire surface of the convolutions, but contralaterally it fully exposes the convolutional crests to the force of contrecoup. Thus, in severer falls, the intracranial pressure situation at the moment of impact considerably differs from that in blows to the movable head and is, in our opinion, the main reason that in falls the main damage is found at the side of contrecoup. Since Friede [5] could already demonstrate a contralateral increase in pressure due to acceleration during mild falls, it may be assumed that with increasing rate of acceleration the resulting intracranial pressures may be quite considerable in magnitude at the moment of impact.

It may be mentioned that occasionally coup lesions, usually underlying a depressed fracture, may be the only cerebral damage in a fall and that, without knowing the circumstances leading to the injury, one may erroneously interpret the lesions as sequelae of a blow. In cases of this kind in our collection, there was very likely no significant preimpact acceleration and the object struck was pointed or edged so that the mass of brain displaced along the line of impact was not large enough to reach the contralateral surface of the brain with traumatizing force. However, in some instances there were herniation contusions in cerebellar tonsils, as were seen in moderately severe blows.

Besides skull flattening or indentation, concomitant shifting of brain tissues, and relative action of positive and negative acceleration pressures, some other physical events which occur in falls and in blows alike should be discussed and appraised in regard to their significance in preventing or furthering contusions. One of them is skull vibration, which Saucerote [27] (1778) already held responsible for contrecoup contusions. According to him, vibrations travel from the point of impact through the skull toward the opposite pole, where they become so forceful by overlapping that the skull fractures and contuses the brain. More recently, Sjövall [28] and his co-worker Anselius offered a modification of the vibration theory. They believe that the sound wave traveling through skull and brain is the most important physical factor for producing contrecoup lesions. If the sound going through the skull is faster than the one going through the cranial content, it supposedly creates an initial negative pressure over the contralateral side of the brain, and only such negative pressure is believed to be able to cause contrecoup lesions by tearing vessels. On the other hand, if the sound traveling through the skull is slower than that going through the brain, the initial contralateral pressure would be positive and would not produce morphologic damage. Two observations strongly speak against this concept: Friede [5] could experimentally demonstrate that any skull fracture

stops propagation of vibrations and, on the other hand, that the combination of skull fracture and contrecoup lesion is a rather frequent finding in human autopsies. Therefore, in cases with skull fracture, vibration of the cranium obviously plays no part in the mechanism of contrecoup contusions. If the skull remains intact, it may contribute to their development, but certainly in a minor way.

Besides vibration, a sufficiently strong blow or fall may cause an elastic deformation of the entire skull with an initial sudden shortening of the line connecting the impact area with the contralateral side and with an outward bending of the portions of the skull lateral to it. This over-all deformation constitutes the first phase of a rapidly dampened gross oscillation of the skull, but only its initial phase has been considered by some authors to be significant for the development of contrecoup lesions. Munro [29] believes that in this way the contralateral skull slaps the brain at the moment when it is still in contact with the skull and that such slapping produces the contrecoup lesions. The over-all deformation most likely occurs in falls on the vertex or on the buttocks and may result, for instance, in a tear of the corpus callosum (Lindenberg et al.[30]). However, in impacts to the perimeter of the skull, the deformation will be of lower intensity because of the resistance offered by firm buttresses. Furthermore, any fracture will minimize the degree of deformation except for impacts to the vertex. Friede,[5] who in his experiments always found an initial negative pressure over the contralateral brain surface in blows to the perimeter of the skull, observed that in blows to the vertex the contralateral initial pressure was always positive. This exceptional situation obviously results first from the elasticity of the calvaria, especially of temporal and parietal bones, being greater than that of the base of the skull, and, second, from the support given by the spinal column to the contralateral portion of the skull. Because of this support, the acceleration of the head in blows to the vertex will generally be less than in blows to other areas of the head, and in falls on the vertex the body weight, or any part of it, will press against the base of the skull and thus facilitate the ellipsoid deformation.

It is, however, questionable how much this deformation really contributes to the development of contrecoup lesions. Since the brain has to follow the outward bending of the temporal portions of the cranium, some of the shifting force will be distracted from the line of impact direction and become absorbed. This absorption will be considerable if the skull is thin and elastic as it is in infants and children. This is, in our opinion, one reason that contrecoup lesions are less frequent in this age group than in adults. In impacts causing little or no deformation of the entire skull, there will be some outward bending of the bone in the vicinity of the area of impact. This more local outward bending, which often leads to radial fractures of the skull, must also have some suction effect on the underlying brain surface and absorb at least some of the impact force, thus helping to prevent the occurrence of coup lesions in falls. This is most likely the case in falls on the back of the head.

One factor influencing the occurrence of contusions is the thickness of the layer of cerebrospinal fluid over the areas under stress. It has often been mentioned that the frequent involvement of orbital and anterior temporal convolutions by contrecoup lesions is partially due to the absence of a significant cushion of cerebrospinal fluid (Spatz,[24] Welte,[25] and others). On the other hand, if more cerebrospinal fluid is present because of some atrophy of the brain, it often reduces the intensity of contusion, or even may prevent these lesions. Whatever the natural thickness of the cushion of fluid over a given region of the brain may be, it will change during acceleration of the head when given time to shift, as it is the case during a fall. A positive acceleration force will reduce, and a negative one increase, it. Therefore, over the area going to be struck in a fall, the cushion will be thicker and provide better protection. Over the con-

tralateral surface of the brain, it will be thinner and therefore promote contusion. Since Brodie[31] (1828), many authors have also pointed out that the frequent involvement of orbital and anterior temporal lobes by contusion is related to the irregularities of the floor of the anterior and middle cranial fossae, due to the so-called impressiones gyrorum. It would be erroneous, however, to believe that these irregularities are responsible for the general distribution pattern of contrecoup lesions. This is primarily determined by the direction of the impact, as mentioned before. The irregularities of the base of the skull, the sharp margins of the lesser sphenoid wings, and the edges of falx and tentorium often promote the occurrence of contusions by locally enhancing the shear strain within the brain tissue; but if the impact is pointed toward the tentorium, for instance, it is not unusual to find contusion hemorrhages, and even contusion necroses, in the cortex of the lower temporal-occipital region facing this dural duplication, while the orbital and anterior temporal convolutions may show no lesions whatsoever. The only difference will be that the production of contusions opposite smooth surfaces of the skull or of the tentorium and falx will need more force than that in the cortex facing bony irregularities or edges.

Finally, those cases should be briefly discussed in which the impact was received while the head was contralaterally supported. This is an exceptional and relatively rare version of head injury, and only very few cases have been properly observed. The consensus seems to be that in such cases coup, as well as contrecoup, lesions are rare except when great violence was involved. This is in keeping with experimental findings by Denny-Brown and Russell,[10] who had to inflict a crushing injury to the supported head in order to produce the same concussion effect as that obtained in milder blows to the movable head. Similarly, Russell[11] reports a case in which the head was crushed between two solid objects without the patient losing consciousness immediately. Undoubtedly, a firm contralateral support of the head changes markedly the physical events taking place at the moment of impact. First, a large amount of the force transmitted to the skull is absorbed not only by any fracturing of the skull but also by the supporting medium; second, in the resting head the cushion of cerebrospinal fluid under the area of impact is thicker than normal because the brain, heavier than spinal fluid, is resting on the contralateral inner surface of the skull; third, the support of the head does not allow any appreciable acceleration, and, therefore, there is no positive acceleration pressure over the brain area underlying the side of impact; fourth, the temporary compression of the skull between the area of impact and the supporting medium results in some outward bending of the perimeter of the skull, which will be followed by the brain and, therefore, distract the shifting force from the line of impact direction; fifth, the pressure exerted on the brain by any contralateral indentation of the skull, furthermore, counteracts shifting force. As the result of all these physical events, there will be no, or very little, shifting of brain tissues in the direction of impact, a fact which would explain why even concussion occurs only if the impact is of a crushing nature. Some coup or contrecoup contusions may be expected to occur if the impact force exceeds a certain magnitude and the consistency of the supporting medium is rather low.

Summary

1. Presence, distribution pattern, and absence of contusions depend not only on degree, site, and direction of force transmitted to the brain, and on physical and anatomic characteristics of the various areas of the skull, but also on whether the head is accelerated by the impact (blow to the movable head) or is not accelerated (blow to the supported head) or is in a state of acceleration at the moment of impact (fall on the head).

2. Regarding blows to the movable head, postmortem findings suggest that the physical changes taking place within the cranium

at the moment of impact differ, depending on the severity of the blow.

(*a*) The absence of coup lesions suggests that the blow accelerated the head but did not produce depression of the skull sufficiently severe to damage the subjacent brain tissues. Because of the inertia of the brain, acceleration of the head produces within the cranial cavity initially positive acceleration pressure over the brain at the site of impact and a negative one over the contralateral brain surface. These pressures and subsequent oscillating movements of the brain may lead to extracerebral bleedings but are not forceful enough to cause cerebral contusions except for "gliding contusions" along the dorsal margin of the cerebral hemispheres.

(*b*) The presence of coup lesions indicates that besides acceleration a significant local depression of the skull took place, sometimes manifested by depressed fracture. The coup lesions are believed to result from combined actions of positive acceleration pressure and sudden positive pressure due to depression of the skull. The latter, furthermore, produces sudden shifting of the brain, which, if strong enough, may reach the contralateral surface of the brain and, by overcoming the negative acceleration pressure, may produce an initial positive pressure throughout. This will prohibit any oscillating movements of the brain; instead, it will force the brain to glide toward the foramen magnum, the area of lowest pressure. "Herniation contusions" in cerebellar tonsils and medulla oblongata are morphologic evidence of such escape mechanism and demonstrate the "vulnerability of the cerebrospinal junction."

(*c*) The presence of coup, as well as contrecoup, lesions indicates that severe skull depression took place, causing not only positive pressure throughout but an exacerbation of this pressure to a traumatizing degree over the area contralateral to that of impact. As indicated by "intermediary coup" lesions, this local contusing pressure is due to the fact that the impulse-like brain displacement is most pronounced beneath the area of maximal skull depression and proceeds along the line of impact direction toward the contralateral side. Therefore, the direction of impact is most essential for determining the site of contrecoup lesions. Since the positive shifting force arriving at the contralateral side will always be reduced by whatever negative acceleration force has developed, contrecoup lesions are, as a rule, less extensive than coup lesions and are often absent in blows to the movable head.

3. In falls on the head, it is assumed that, because of the inertia of the brain during the fall, a negative acceleration pressure develops over the brain next to the site of impending impact and a positive one over the contralateral side, both pressures affecting the thickness of the layer of cerebrospinal fluid. This intracranial pressure condition and the intensity of the fall influence the events taking place in the cranium at the moment of impact.

(*a*) Absence of coup and contrecoup lesions suggests that no or insignificant skull depression occurred. The sudden deceleration of the skull upon impact will cause the brain to "fall through," which event will be followed by a rapidly dampened oscillation of the brain within the cranial cavity. As in mild blows, such movements of the brain may cause extracerebral bleedings and "gliding contusions."

(*b*) If coup and no or very few contrecoup lesions are present, it indicates that a more circumscribed skull indentation, often leaving a depressed fracture, exerted pressure on the subjacent brain tissues exceeding the negative acceleration pressure present and causing a sudden displacement of brain along the line of impact direction, as evidenced by "intermediary coup" lesions. There will be no "falling through" of the brain. Because of positive acceleration pressure still being present over the contralateral side of the brain, there will be a positive pressure throughout the cranial cavity, forcing the brain to glide toward the foramen magnum, as may be evidenced by "herniation contusions" of the cerebellar tonsils. Contrecoup contusions signify that the shift-

ing force reached the contralateral brain surface before the brain had a chance to "fall through." Positive acceleration force still present will enhance the shifting force. The distribution pattern of lesions in these cases is essentially the same as in severe blows to the head.

(*c*) If, as in most cases, contrecoup lesions are extensive and coup lesions are absent or small, it is suggested that a depression, usually flattening of a larger part of the skull, took place at the site of impact, exerting pressure on the subjacent tissue, which, reduced by the negative acceleration pressure present, caused no or little coup injury. Nevertheless, it increased the intracranial pressure and shifted the brain predominantly along the line of impact direction toward the contralateral side. Here the shifting pressure enforced by the positive acceleration pressure caused contrecoup lesions. "Intermediary coup" lesions, seen most frequently in falls on forehead and vertex, prove that mass shifting along the line of impact direction takes place.

4. In blows to the supported head, contusions, especially coup and contrecoup lesions, are very rare, mainly because no significant acceleration develops and most of the positive shifting force is laterally distracted, since the brain must follow simultaneous outward bending of the perimeter of the skull when the points of impact and support suddenly approximate.

5. If in both—blows to the movable head and falls—the vertex is the site of impact, such outward bending may occur predominantly in the temporal regions, causing "stretching contusions" in the corpus callosum. In infants and children, similar outward bending may result from impact to any area of the skull, because of the greater elasticity of the bones, and is believed to be one essential reason why coup and contrecoup lesions are less frequent in this age group than in adults. In the latter, a local outward bending of the skull next to the area of skull depression, which often leads to radiating fractures, will absorb some force transmitted to the brain and, therefore, help prevent coup contusions in falls and restrict the area of coup lesions in blows.

6. The distribution pattern of coup, intermediary coup, and contrecoup contusions is directly related to the impact and its direction. All other contusions—"herniation," "gliding," "stretching," and "fracture" contusions—are indirectly related to the impact, and their location is not necessarily dependent on the direction of impact.

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Acute Tubular and Glomerular Lesions in Rat Kidneys After Uranium Injury

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Introduction

This is a report of changes seen by light microscopy in rat kidneys after acute uranium injury. It is part of a systematic investigation of morphologic alterations of the kidney in physiologic and pathologic states.[5, 6, 14] In this present report the light-microscopic appearance of a lesion involving centrolobular cells is presented and a new finding of the appearance of collagen in this lesion is noted.

Cells of proximal convoluted tubules are found to develop a greater number of various cytoplasmic bodies, which stain like material in the lumens of tubules, than has previously been described by others. These observations add evidence for the special nature of cells in centrolobular regions of glomeruli and for understanding the mechanisms of tubular resorption.

Materials and Methods

To determine effects of different doses of uranium, a group of 16 Sprague-Dawley rats, weighing 70 to 80 gm. was used. Uranium nitrate hexahydrate, $UO_2(NO_3)_2 \cdot 6H_2O$ (Allied Chemical Co., reagent grade), was injected subcutaneously as a 0.6% solution in saline. A dose of 14.4 mg/kg. was found suitable for producing extensive renal lesions. It was given to 26 rats; 18 rats received an equal volume of saline, and 6 were untreated. Urine from rats kept in individual metabolism cages was collected daily under toluene. Urine volumes were recorded daily, and protein was estimated (0 to 4+) using sulfosalicylic acid.[9]

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Animals were killed after 18, 24, and 36 hours, and 2, 3, 4, 5, and 6 days. The rats were anesthetized with pentobarbital (Nembutal). Blocks of kidney were fixed in Zenker-formol and processed according to a method previously described.[8] Paraffin sections were cut at 2μ and stained with a modification of the Masson trichrome stain for mitochondria and other cytoplasmic structures[8]; with erythrosin orange-toluidine blue for cytoplasmic basophilia[10]; with periodic acid-Schiff stain (PAS) counterstained with iron hematoxylin, orange G, and anilin blue, for hyaline droplets and for distinguishing basement membranes and collagen; with phosphotungstic acid hematoxylin of Mallory (PTAH) for fibrin and collagen; with the Verhoeff-Van Gieson procedure for elastic fibers and collagen; with hemalum-phloxine-saffron (HPS) for collagen,[4] and with hematoxylin and eosin.

Observations

After injection rats appeared normally active until 12 to 24 hours before dying, when they huddled in the corner of their cages. Death usually occurred on the fifth day after injection, unless animals were killed earlier. They gained little or no weight, in contrast to controls, which gained regularly. Urine volumes in the control period ranged from 2 to 5 (average 3.5) cc. per day. A summary of the observations is given in the Table in order to facilitate correlation of these lesions with time. There was little or no change in urine protein or volume for the first day after injection. Urine volumes reached a maximum average of 12.9 cc. on the third day and decreased thereafter. Proteinuria became maximal on the fourth day. Grossly, kidneys became pale or mottled after the second or third day.

In *glomeruli* the most prominent lesion is that formed by large centrolobular deposits (Fig. 1). These deposits or masses,

Acute Changes in Rat Kidneys with Uranium Poisoning *

Days After Injection	Control	1	2	3	4	5	6
Urine							
Volume, av. (cc/day)	3.5 †	3.9 ‡	9.1	12.9	5.5	2.9	3.9 §
Protein, av. concent.	1 ‖	1 ¶	3	3	4	4	4
Gross pallor of kidneys	0	0	2	4	4	4	4
Glomeruli							
Centrolobular deposits	0	0	0	1	3	3	4
Vacuolation of epithelium	0	0	0	1	4	4	2
Hyaline droplets in epithelium #	0	0	0	1	3	4	1
Capsular space							
Hyaline droplets	0	0	0	0	2	4	2
Large globules	0	0	0	0	2	4	2
Amorphous material	0	0	0	4	4	4	4
Proximal convoluted tubules							
Vacuolation	1	4	2	1	1	1	1
Necrosis	0	1	2	2	4	4	3
Regeneration	0	0	1	2	3	3	4
Hyaline droplets **	1	1	4	4	3	4	4
Cytoplasmic bodies	1	1	1	3	4	4	4
Casts ††	0	1	4	2	2	3	1
Distal convoluted tubule							
Casts	0	1	2	2	3	4	2
Papillary ducts							
Casts	0	1	2	4	4	4	4
Pelvic epithelium							
Vacuoles	0	0	0	1	3	4	4

* Lesions or structures are graded 0 to 4 on the basis of frequency or size. The maximal degree of development of each lesion is indicated by 4.

† Average of 28 rats.

‡ Average of 13 rats at beginning of experiment.

§ Urine volume was obtained on only one animal.

‖ Average of 17 rats.

¶ Average of five rats at beginning of experiment, of three rats on the fifth day, and of two rats on the sixth day.

Hyaline droplets in both visceral and parietal epithelium.

** Hyaline droplets in proximal tubules in central layer of renal cortex.

†† Casts in proximal tubules in outer layer of renal cortex.

staining deeply with the trichrome procedures, appear in most glomeruli of all rats from the third day on. These lesions are found in varying sizes and numbers, frequently several to a glomerulus. They are especially prominent after the fourth day. They tend to lie in the center of the glomerular lobules between basement membranes or between endothelial nuclei and basement membranes. The blue deposits extend along

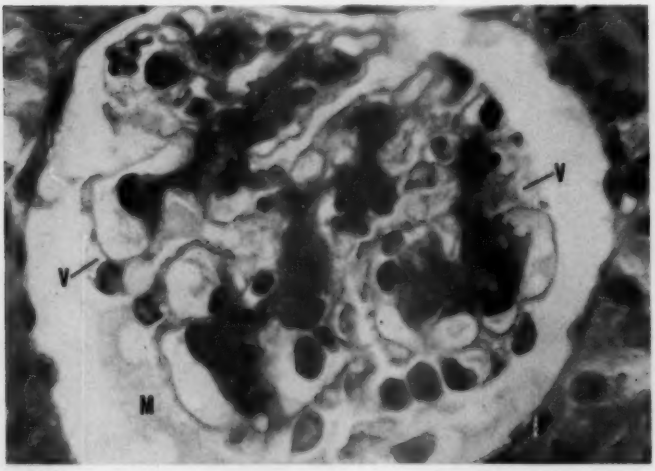

Fig. 1.—Glomerulus six days after uranium injection, showing the centrolobular position of the blue masses, containing rough fibers and appearing dark in the photograph. Some nuclei are intimately associated with these masses. Vacuolation of visceral and parietal epithelium (*V*) is present, as well as an amorphous material (*M*) in the capsular space. Masson's trichrome stain; reduced 25% from mag. × 1,100.

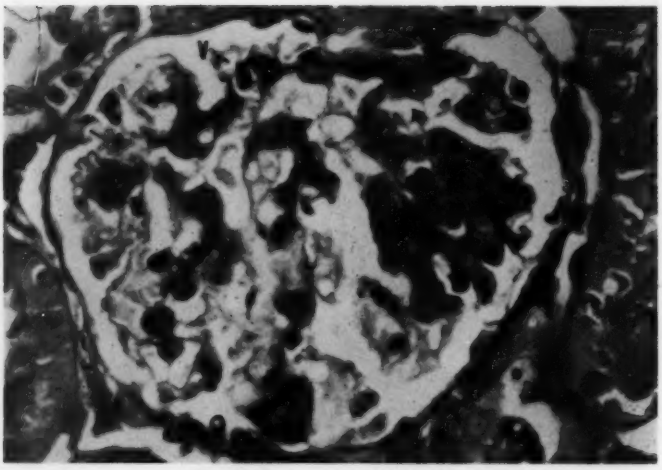

Fig. 2.—Glomerulus five days after uranium injection, showing the centrolobular position of the PAS-positive deposits. Some of the nuclei are intimately associated with these masses. Vacuolation of visceral and parietal epithelium (*V*) is present. PAS-trichrome stain; reduced 25% from mag. × 1,000.

the basement membranes toward the periphery of lobules. At no place can the deposit be identified as lying within the capillary lumen, and no evidence for definite capillary obstruction is seen. Areas involved may be irregular and reach 15μ or more in diameter. The lesion is composed of intermingled blue-stained fibrillar or stringy material. The individual fibers have an irregular outline up to 0.5μ wide and are interspersed with blue granules. In some places these fibers are interspersed with fine material staining pink like cytoplasm. Larger lesions may contain one or more irregularly shaped nuclei with an extremely thin, pink-stained cytoplasmic covering. This may appear as a clear zone around the nucleus. The nature of centrolobular intercapillary cells is not clear. They could be either endothelial cells or the so-called mesangial cells. Less frequently, intercapillary cells with a foamy cytoplasm may also be seen. The space between the blue fibers appears empty or light blue. These fibers stain like collagen with all stains used and not like basement membrane, elastic fibers, or fibrin.

In addition to blue masses, the centrolobular area may show some focal homogeneous, granular, or fibrillar PAS-positive deposits, reaching up to 15μ in diameter (Fig. 2).

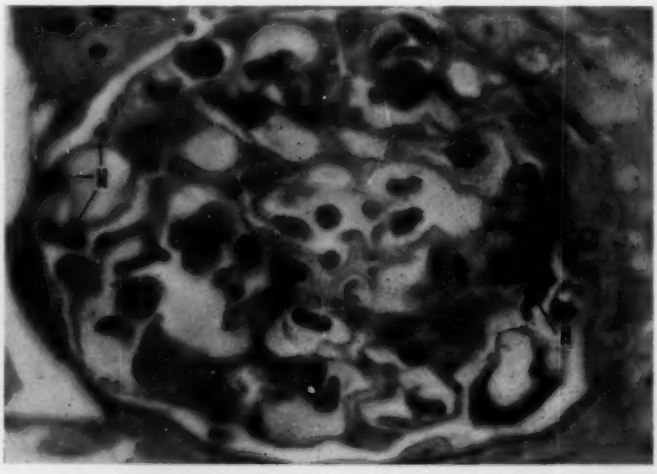

Fig. 3.—Glomerulus from a rat four days after uranium injection. Most epithelial cells contain several hyaline droplets (*H*), varying in size and occasionally forming groups. PAS-orange G stain; reduced 25% from mag. × 1,400.

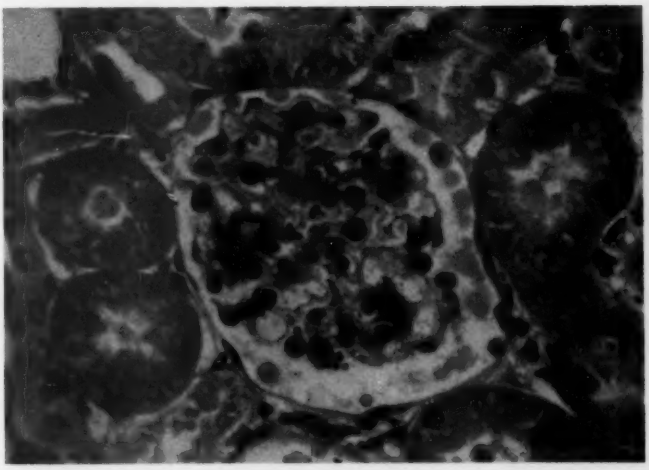

Fig. 4.—Glomerulus five days after injection of uranium. Large globules are apparent in the glomerular space and in surrounding proximal convoluted tubules. Hyaline droplets are also seen in the glomerular epithelium. Masson's trichrome stain; reduced 25% from mag. × 840.

Occasionally material staining with orange G may be associated with the PAS-positive deposits. These deposits may lie around the capillary lumen or even fill it and incorporate erythrocytes, thus resembling hyaline thrombi. PAS-positive deposits are less frequent than the blue masses and are usually found in glomeruli of the inner cortical layer. The PAS-positive deposits are less prominent in those glomeruli which have small blue masses. It is difficult to determine the exact anatomical relationship of the PAS-positive basement membrane to the PAS-positive deposits. No change is detected in clearly recognizable endothelial cells.

Extensive vacuolation of epithelial cells occurs after the third day, with some vacuoles reaching diameters of 10μ or more. Contents of vacuoles may be clear or stain pale blue (Figs. 1 and 2). Hyaline droplets are seen frequently in visceral and less frequently in parietal epithelial cells from the third day on (Fig. 3).

In the *capsular space*, hyaline droplets are found free from the fourth day on. Large round, slightly granular globules up to 10μ in diameter are also found free in the capsular space from the fourth day on (Fig. 4). Staining from pale pink to pale blue, they frequently lie in groups. The

Fig. 5.—Renal cortex five days after uranium injection. Necrosis is most extensive in the middle layer of the cortex. Cells of the proximal tubules in the inner cortical layer are markedly swollen. Casts are seen in all layers. Masson's trichrome stain; reduced 25% from mag. × 70.

globules are often found in tubules adjacent to glomeruli. The capsular spaces frequently contain various amounts of an amorphous light-blue material (Fig. 1), and occasionally red blood cells are seen from the third day on.

Proximal convoluted tubules are the most severely involved segments of the nephron. After 18 to 24 hours a slight difference can be seen between the kidneys of treated and of control rats with Masson's trichrome stain. At this time injured animals show occasional necrotic cells in proximal convoluted tubules. Necrosis in them is of a pyknotic type, and the number of cells showing it increases sharply with time, becoming maximal on the fourth day in the portions of the proximal tubules situated in the central cortical layer (Fig. 5). Necrotic cells appear as almost completely autolyzed masses within the lumen, leaving basement membranes bare.

In the same period, and especially after the third day, many cells of the proximal tubules in the inner cortical layer become markedly swollen and lose mitochondria and cytoplasmic boundaries. Nuclei enlarge and become faint, even with toluidine blue staining. This type of change has been considered to represent karyolytic necrosis.[2] Scattered among these cells are some pyknotic ones. Some groups of karyolytic cells fill the lumen of the tubule and rest over a continuous layer of low basophilic cells with frequent mitoses. Such basophilic cells, frequently found from the third day on, are generally considered to be regenerating. Pyknotic necrosis also involves some of the proximal tubules in the outer layer of the cortex. Large vacuoles are most prominent in the cytoplasm of proximal tubule cells of rats killed one day after injection. Hyaline droplets increased in prominence on the second day in cells of the proximal tubules, especially in the central layer. Various other irregular cytoplasmic bodies normally present, and staining blue, red, brown, or black, increase markedly in size and number in the injected animals, particularly on and after the third day (Fig. 6). Homogeneous and granular casts, staining blue, red, brown, or black with trichrome stains, are prominent in proximal tubules of the outer cortex between two and five days after injection.

In cells of *distal tubules* no significant changes are seen. Red- and blue-staining casts, however, are found in lumens in 24 hours, and these become more frequent from the second day on. In *papillary ducts* dark-red, inspissated casts appear on the first day and become more numerous from the third day on.

Pelvic epithelial cells covering the base of renal pyramids frequently show a few small

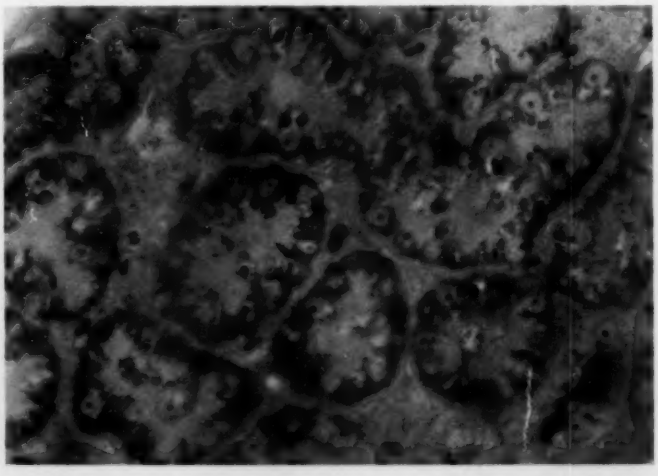

Fig. 6.—Proximal convoluted tubules six days after uranium injection, showing numerous cytoplasmic bodies. The bodies are round, irregular, or elongated, and vary in size up to 5μ in diameter. Many are partially surrounded by a clear space. The darkly staining bodies can be easily distinguished from the lighter rod-like mitochondria. PTAH stain; reduced 25% from mag. × 870.

clear vacuoles. Three days after uranium injection the number and size of these vacuoles increase considerably. Moreover, they come to contain a homogeneous material, staining blue with a trichrome stain.

Comment

The present experiment developed during the course of a systematic investigation of changes in the kidney under physiologic and pathologic conditions. Uranium poisoning was selected for study because the morphologic and physiologic changes occurring in kidneys in this condition have been extensively documented.[2,7,15,16] However, in order to correlate ultrastructural and functional alterations, it was necessary to determine the time and location of such changes in our experimental animals. This report emphasizes only those changes which have not been previously described, or which have not received much attention.

Although earlier investigators[7] described several changes in glomeruli after uranium injection, recent workers[2] have described only minor or transitory changes in glomeruli. The present observations are in general agreement with those reported by the earlier investigators.

The centrolobular collagenous deposits have not been described previously in relation to uranium. The closest counterpart, either in experimental or in naturally occurring renal disease, seems to be lobular glomerulonephritis.[1] The appearance of the centrolobular deposits after three days correlates better with proteinuria than with polyuria. Because the blue deposits, particularly if small, are sometimes associated with PAS-positive material, the possibility exists that a mucopolysaccharide-like material may precede the abnormal collagenous deposit. The staining procedures used show that collagen is not normally present in glomeruli. The appearance of collagen in the centrolobular part of glomeruli indicates that cells in this area are different from endothelial cells, as has been suggested by others.[12,17]

Because hyaline droplets are common in glomerular epithelium when proteinuria is maximal, they may indicate protein resorption similar to that of the proximal tubules. There is evidence from other work using labeled proteins that protein can be taken up by glomerular epithelium.[8] The presence of hyaline droplets and large globules in capsular spaces suggests that they may come from epithelial cells, either by disintegration or by potocytosis, similar to that observed in tubule cells.[13] The blue material in the capsular spaces probably is a consequence of the increased permeability of the glomeruli.

The present study confirms the morphologic changes described by others in the renal tubules, as well as progressive changes in urine volume and in proteinuria.[2,7,15,16] The significance of large vacuoles appearing early in cells of proximal convoluted tubules is unknown. There has been considerable evidence relating the appearance of hyaline droplets in proximal convoluted tubules to proteinuria under certain conditions.[11] The prominence of cytoplasmic bodies staining differently from hyaline droplets has not been emphasized in the past. The appearance of these cytoplasmic bodies at the time when casts in the proximal tubules become less prominent suggests the possibility that these bodies may be formed, at least in part, by resorption of material from the lumen of tubules. This concept is supported by the observation that the bodies have as many different colors as are found in the casts with the different stains used. The appearance of casts in the collecting tubules after several days suggests an obstructive factor for the terminal oliguria.

Summary

Glomerular and tubular changes in acute uranium poisoning in rats have been correlated with polyuria and proteinuria occurring in these animals.

The most striking change is a large centrolobular lesion developing in all glomeruli after the third day. This lesion is composed of fibers staining like collagen.

Basement membranes adjacent to small lesions sometimes show varying amounts of a PAS-positive material. The formation of collagen in the centrolobular area suggests the existence of an intercapillary cell different from endothelial cells.

The glomeruli of uranium-treated animals also show vacuolation and hyaline droplets in epithelial cells. The capsular space contains hyaline droplets, large globules, and an amorphous material.

Marked changes in the tubules, such as necrosis, regeneration, hyaline droplets, and casts, are similar to those described by others. The increase of cytoplasmic bodies in proximal convoluted tubules while casts become less prominent suggests that these bodies may be formed by resorption of material from the lumen of tubules.

Dr. Sidney C. Madden, Department of Pathology, Medical Center, University of California, Los Angeles (24).

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News and Comment

ANNOUNCEMENTS

U.S. Department of Health, Education, and Welfare, Public Health Service.—A sum of $1,500,000 has been appropriated by the Congress of the United States for fiscal year 1960 to further the widespread application of existing knowledge of preventing and controlling cancer. These funds will be administered by the Cancer Control Program, Public Health Service, under the technical guidance of the director of the National Cancer Institute.

Guide lines for the use of this money were developed by the Cancer Control Program's newly formed Advisory Committee, which includes Dr. David Wood, past-president of the College of American Pathologists, and Dr. Joseph A. Cunningham, member of the Board of Governors of the College of American Pathologists. The 12 other physicians and surgeons comprising the Advisory Committee are Dr. Ulrich Bryner, Dr. Bernard Bucove, Dr. John W. Cline, Dr. Warren H. Cole, Dr. Harold S. Diehl, Dr. Lloyd M. Graves, Dr. John P. Lindsay, Dr. James J. Nickson, Dr. Mack I. Shanholtz, Dr Charles E. Smith, Dr. John W. Spellman, and Dr. Samuel G. Taylor III.

The Cancer Control Program and its Advisory Committee believes that the best opportunities for demonstrating better ways of providing community cancer control services at this time lie in the following:

1. Professional and technical education in cytology
2. Screening female beneficiaries of medical care for cancer of the cervix
3. Selected educational projects, particularly public information and follow-up services, to emphasize the importance of periodic uterine cytologic examinations
4. Professional educational activities emphasizing the importance of including diagnostic aids in complete health examinations
5. Selected public educational projects on the desirability of and need for examinations that maintain health
6. Evaluation of effectiveness of public educational activities
7. Tumor registers collecting data of exceptional value
8. Extension and evaluation of rehabilitation programs (in cooperation with State rehabilitation agencies)
9. Selected programs demonstrating effective treatment for cancer in public beneficiaries of medical care

The types of projects suggested are not meant to exhaust all possibilities. Other worth-while, locally sponsored, and locally directed demonstration projects will be considered.

Applications which are acceptable from nonprofit organizations and institutions, as well as official health agencies, are reviewed in much the same way as requests for research grants at the National Institutes of Health. The Advisory Committee to the Cancer Control Program and the National Advisory Cancer Council reviews the applications and recommends their approval or disapproval. Acting on these recommendations, the chief of the Bureau of State Services, Public Health Services, takes formal action.

Additional information may be obtained from the Cancer Control Program, Division of Special Health Services, Department of Health, Education, and Welfare, Washington 25, D.C.

Pan-Pacific Surgical Association.—The Eighth Congress of the Pan-Pacific Surgical Association will be held in Honolulu, Sept. 27 through Oct. 5, 1960. Information concerning the meeting may be obtained by writing to Dr. F. J. Pinkerton, Director General of the Pan-Pacific Surgical Association, Suite 230, Alexander Young Building, Honolulu 13, Hawaii.

National Vitamin Foundation.—The National Vitamin Foundation of New York announces an all-day symposium on the Genetic Control of Biochemical Processes in Diseases of Metabolism, to be held at the Jewish Hospital of St. Louis, St. Louis, on Friday, Oct. 7, 1960. The symposium will be under the joint sponsorship of the National Vitamin Foundation, the Jewish Hospital of St. Louis, St. Louis University School of Medicine, and Washington

University School of Medicine. Further information may be obtained from William Rubin, Director of Public Information, the National Vitamin Foundation, Inc., 149 E. 78th St., New York 21.

PERSONAL

Dr. Valy Menkin's Appointment.—Dr. Valy Menkin has been appointed Professor of Pathology and Chairman of the Department of Pathology at the University of Kansas City School of Dentistry, Kansas City, Mo., beginning Feb. 1, 1960.

Dr. Wilhelm C. Hueper Wins Ann Frankel Rosenthal Memorial Award.—Dr. Wilhelm C. Hueper was given the Ann Frankel Rosenthal Memorial Award at the meetings of the American Association for the Advancement of Science in Chicago in December, 1959. The award was presented in recognition of his work on the causes of certain occupational cancers.

Dr. Valy Menkin Awarded Pasteur Institute Medal.—Dr. Valy Menkin, guest investigator at the Henry Phipps Institute of the University of Pennsylvania, was recently awarded the Pasteur Institute Medal and also a medal from the University of Liége. He received these while serving in Europe as a visiting professor under the Fulbright program.

Dr. C. J. Van Slyke Retires.—Dr. C. J. Van Slyke, deputy director of the National Institutes of Health, has retired, but will continue to function as a consultant. Pathologists will always be mindful of the contributions of Dr. Van Slyke to the betterment of pathology through his services in the National Institutes of Health in the development of the research grants and Study Section programs, which have played such an important part in the furtherance of biological research in the past decade and a half.

DEATHS

Dr. Earl W. Cauldwell Dies.—Dr. Earl W. Cauldwell, of Beloit, Wis., died on Oct. 3, 1959, at the age of 43. Dr. Cauldwell had been an assistant professor of pathology at the University of Illinois College of Medicine and was associate professor in Beloit College, being responsible for the development of a hospital-college laboratory technicians' training school.

Books

A Text on Systemic Pathology. Volume II. Edited by Otto Saphir, M.D. Price, $38.00. Pp. 1085, with 979 illustrations. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1959.

This is the second book of a two-volume text. The first volume was reviewed in the February, 1959, issue of the A.M.A. ARCHIVES OF PATHOLOGY. The text was written in an attempt to provide both medical students and hospital pathologists with a ready source of specialized information. It was planned to be the work of a single author, and the first volume was written almost entirely by Dr. Saphir. However, because of the amount of time required to prepare a book of this magnitude, he has called upon several of his former students and associates to contribute sections to the second volume.

Dr. Saphir has written the chapters on the alimentary tract, oral cavity and related structures, liver, pancreas, and peripheral nerves. Paul Szanto collaborated with Dr. Saphir on the chapters dealing with the nose and ear. Lorenz Zimmerman wrote the section on the eye; Max Appel, that on endocrine glands, Martin Swerdlow, that on skin; George Amromin, that on bones and joints; Arthur Rubenstone, that on skeletal muscle, and Jacob Chason, that on brain, meninges, and spinal cord.

An attempt has been made to relate pathologic anatomy to the altered function. However, this remains primarily a text of pathologic anatomy. It is indeed a monumental work.

A Textbook of General Physiology. Second Edition. By Hugh Davson, D.Sc. Price. $14.50. Pp. 845, with 384 figures. Little, Brown & Company, 84 Beacon St., Boston, 1959.

The second edition of this fine book by Hugh Davson is much to be welcomed. Considerably larger than the first edition, the book retains the same central organization proceeding from a consideration of (1) the structural basis of living matter, through (2) transformation of energy in living systems; (3) transport of water and solutes; (4) characteristics of excitable tissues; (5) mechanism of contraction of muscle, and (6) light, its effect on and its emission by the organism.

Each of these sections has been carefully brought up-to-date, and three new chapters have been added to Section 4, i.e., (*a*) sensory response; (*b*) excitability of cardiac muscle, and (*c*) electrical activity in smooth muscle.

This plan of organization is a reasonable one and does not differ markedly from that of other successful texts. However, each section, indeed chapter, tends to be exhaustive, self-contained, and typically sophisticated in its treatment. This is a feature which seems to encourage repetition. Moreover, the elementary student meets from the outset some rather "difficult" topics.

It is possible to cavil in regard to either the selection of topics or the title of the book, which leads one to expect a broad coverage of the field. According to the author, this is "the study of those features of life which appear to be common to all forms." In actuality, many topics which, to this reviewer, seem properly to fall within the purview of general physiology thus defined are scarcely touched. Thus, there is little or no treatment of such topics as nutrition; cellular metabolism, its organization and regulation; physiological genetics; growth and differentiation, and various other integrative mechanisms. On the other hand, there is considerable discussion of such subjects as cerebrospinal and ocular fluids, absorption from the intestine, and kidney function, which, however interesting they may be as particular examples of general principles, are hardly features of life common to all forms.

As must be true of all texts, some sections of this book are stronger than others; particularly to be commended are the treatments of permeability and of excitation.

"A Textbook of General Physiology" by Hugh Davson is a sophisticated treatment of selected topics of general physiology, particularly useful for advanced courses in general and cellular physiology and as collateral reading for well-prepared and interested students of mammalian physiology.

Cellular and Humoral Aspects of the Hypersensitive States. Edited by H. Sherwood Lawrence, M.D. Price, $18.00. Pp. 667, with 120 figures. Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 49 E. 33d St., New York 16, 1959.

This volume is the proceedings of a symposium held at the New York Academy of Medicine to consider the direction and tempo of changes that have evolved in the past decade in immunological concepts. Experimental observations of diverse phenomena, such as cellular origins, acquired immune tolerance, and allergic encephalomyelitis, by investigators in various disciplines have given rise to an entirely different concept of the nature and scope of immunology than existed when the immune aspects of infectious disease were dominant. The articles included in this book are summaries of the current state of knowledge and concepts. Each author has been allowed to present his topic in detail, and the discussions following each chapter add to the presentation. While emphasis is on clinical and experimental hypersensitivity, many problems of basic immunology are also considered.

Modern Trends in Pathology. Edited by D. H. Collins, O.B.E., M.D.(L'pool), F.R.C.P. (Lond). Price, $15.00. Pp. 346, with 135 figures. Paul B. Hoeber, Inc. (Medical Book Department of Harper & Brothers), 49 E. 33d St., New York 16, 1959.

A wide variety of interesting but seemingly unrelated topics are included in this volume. The basis for inclusion by Douglas T. Collins, the editor, is that the work "illustrates either pathology in general or major fields in which interest is widespread." Many important areas of current research are covered. Nearly all of the techniques employed by pathologists in the search for understanding of disease have been used in the studies described by the various writers.

Sir Roy Cameron discusses ultrastructural and biochemical alterations in "The injured cell." R. J. V. Pulvertaft describes his experience with microscopic "Examination of pathological tissue in a fresh state." "Injury to connective tissue" is considered by D. M. Angevine. C. L. Oakley reviews "The localization of antibody production." "Endogenous mechanisms in the acute inflammatory reaction" are outlined by W. G. Spector. The effects of "Whole body irradiation" are discussed by R. H. Mole. R. A. Willis describes "Some uncommon and recently identified tumors." J. N. P. Davies presents the results of studies on "Cancer in Africa." The problem of "Cancer of the liver" is discussed by P. E. Steiner. "Disorders arising from the tropoblast" are considered by W. W. Park. G. Payling Wright outlines the current ideas on "Movements of neurotoxins and neuroviruses in the nervous system." A. C. P. Campbell discusses "The pathological relationships of 5-hydroxytryptamine." "The human adrenal cortex in disease" is described by T. Symington. J. Gough considers current concepts of "Occupational pulmonary diseases." The problem of "Pathological ossification and osseous metaplasia in man" is discussed by D. H. Collins and R. C. Curran.

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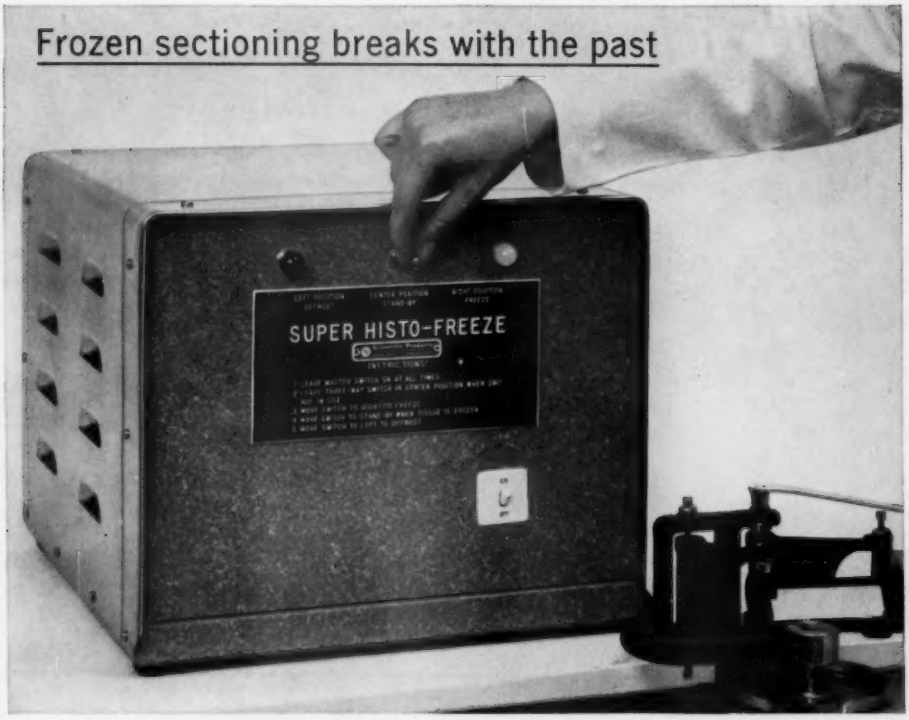

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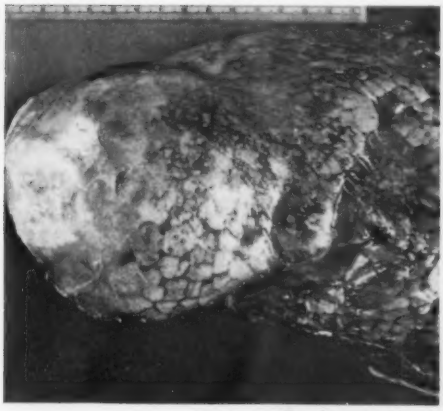
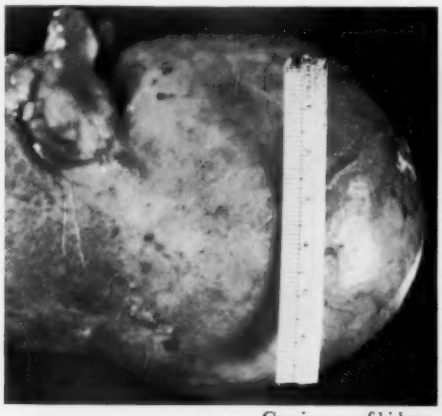


Carcinoma of lung



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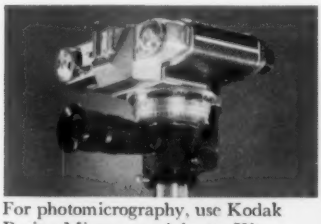

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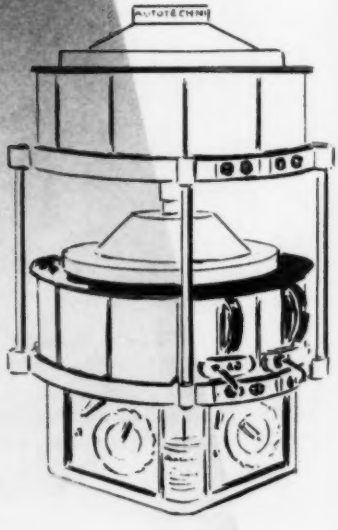

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